Bulgarian Soil Science Society

BULGARIAN JOURNAL OF SOIL SCIENCE®

2021 Volume 6 Issue 1 e-ISSN: 2367-9212 ISSN: 2534-8787

BULGARIAN JOURNAL OF SOIL SCIENCE®

(Peer Reviewed Open Access Journal)

Published by Bulgarian Soil Science Society

Editor-in-Chief
Prof. Metodi Teoharov

Executive-Editor
Prof. Irena Atanassova

Editorial Board

Prof. Rattan Lal
Prof. Rainer Horn
Prof. Evgeny Shein
Prof. Pandi Zdruli
Prof. Milan Mesič
Prof. Dimitranka Stoycheva
Prof. Zornitza Popova
Prof. Radka Donkova
Prof. Ludmila Malinova
Prof. Veneta Krasteva
Assoc. prof. Zdravka Petkova
Assoc. prof. Rada Povova
Assoc. prof. Milena Kercheva
Assoc. prof. Neli Gadzhalska
Assist. prof. Yonita Perfanova

Technical Editor - Pre-print - Web Support
Assoc. prof. Biser Hristov

The Executive Board of the Bulgarian Soil Science Society has decided to begin publishing Bulgarian Journal of Soil Science in relation to the International Year of Soils 2015, as a web-based, free, and open-access international scientific journal. The editorial advisory board consists of internationa researchers and specialists in the field of soil science and other related sciences.

We are confident that Bulgarian Journal of Soil Science will unite the research of specialists and scientists in soil science from all over the world, as well as help them in the processes of their scientific integration. Bulgarian Journal of Soil Science is an international open-access, web-based scientific journal dedicated to the publication and discussion of high-quality research in the field of soil sciences. Bulgarian Journal of Soil Science publishes scientific research that contributes to the understanding of soil and its interaction with humans and the entire earth system. The scope of the journal includes all topics relevant to the study of soil science as a discipline. The journal publishes original papers in specific topic sections on global and regional, theoretical and experimental studies concerning the problems of genesis, geography, physics, chemistry, biology, fertility, management, conservation, and remediation of soils.

Author instructions can be found on the official webpage and in the journal.

http://www.bsss.bg/Journal.html **e-mail**:journal@bsss.bg **e-ISSN: 2367-9212 ISSN: 2534-8787**

BULGARIAN JOURNAL OF SOIL SCIENCE®

CONTENTS

VOLUME : 6 ISSUE : 1 PAGES : 1 – 103

Soil Genesis and Classification

1 Genesis of Soils with Anthropogenic Influences and their Classification. **3 – 8**
Metodi Teoharov, Ivaylo Kirilov

Biological Factors and Soil Biodiversity

2 Influence of the Red Wood Ant *Formica lugubris* Zetterstedt (Hymenoptera: Formicidae) on the Surrounding Forest Soil Influence of the Red Wood Ant on the Forest Soil. **9 – 17**
Angelos Tsikas, Paraskevi Karanikola, Michail Orfanoudakis

Soil Reduction Potential and Sorption Properties

3 Ionic Mobility of Cations as Affected by Redox Status of Two Different Soil Textures. **18 – 32**
G.O. Dayo-Olagbende and B.S Ewulo

Humus Formation and Soil Organic Matter

4 Influence of Forest Vegetation on Organic Matter Composition after Restoration of Mining Spoils in the Town of Pernik. **33 – 41**
Vania Kachova

Agrotechnics and Parameterization of Soil Conditions

5 Influence of Agro-Technical Treatments on Some Physical and Agrochemical Parameters of Haplic Vertisols. **42 – 58**
Vanya Lozanova, Ivan Dimitrov

Irrigation and Soil Degradation

6 Erosion Processes Due to Sprinkler Irrigation **59 - 64**
Nelly Gadjalska, Rossitza Petrova, Snejan Bozhkov,Viktoriya Kancheva

Design and Experimental Fertilization

7 Assessment and Analysis of Designs for Fertilizer Experiments **65 – 77**
Alexander N. Sadovski

Manure and Soil Protection

8 The Effects of Chicken Manure Digestates on the Removal of Diesel Range Organics from Petroleum Products Polluted Soils **78 – 95**
S. U. Oghoje, J. E. Ukpebor, E. E. Ukpebor,

In Memoriam

Prof. Dr. Ivan Atanasov Petkov **96 – 97**

Anniversary

Prof. Dr. Rayna Dilkova, at 90th Years Old **98 – 99**

Instructions for Authors **100 – 103**

Genesis of Soils with Anthropogenic Influences and their Classification

Metodi Teoharov, Ivaylo Kirilov
"N. Poushkarov" Institute of Soil Science, Agrotechnologies
and Plant Protection,
7 Shosse Bankya Str., Sofia 1331, Bulgaria
Corresponding Author: Metodi Teoharov, e-mail: mteoharov@abv.bg

Received: 9 December 2020 *Accepted: 10 March 2021*

Abstract

The genesis of anthropogenic soils is widely studied. Soil changes or the formation of new soil types due to human activities are a basic and major factor about anthropogenic soils. A genetic-diagnostic characteristics of anthropogenic influences and the current processes, which influence the formation of specific by composition, properties and structure soils, has been performed. Generally human activities and the consequent running processes are of a different nature and the reflection of these activities on soil cover is durable and visible. All that provides grounds for anthropogenic soils to be grouped and systemized as a separate soil type with subtypes and to be correlated according to according to FAO diagnostics and classification (2014) and the accredited criteria for soil assessment in the world referential base.

Key words: anthropogenic soils, genesis, diagnostics, classification

Introduction

Anthropogenic soils have been recently formed or heavily modified natural soil formations as a result of human activities. They are poorly researched in our country and in the world as a whole. Anthropogenic influences and processes, which are observed only in agricultural activities (agro-technical, agro-chemical, plant protective, hydro-ameliorative, ameliorative, irrigational and agro-ecological) lead to profound changes in their initial properties and characteristics. At the same time, it is possible to enhance the soil properties and to increase their productivity by well-performed ameliorative activities. In the national classification they are defined as Agrogenic soils on second taxonomic level (Penkov, et al, 1992). In the latest national classification of Bulgaria are examined as independent soil type Anthrosols (Teoharov et all, 2019). In the World Reference Base of soil resources (2014) also are definite as Anthrosols.

Results and Discussion

Genetic-diagnostic and Processing Characteristic of Soils

These soils are spread locally in the whole country and their morpho-genetic characteristics is proved up to establishing and determining their existing peculiarities or problems. On this basis they are separated from the normally developed soils, their anthropogenic load is investigated and the functions which reproduce their secondary genesis are improved. They differ essentially from initially formed soils, while the modern (anthropogenic) soil-formation process forms new diagnostic horizons (anthric, anthraquic) in the course of time. The large variety and their territorial partition provide the opportunity of general characterization of the conditions of their formation in the framework of the soil type (Penkov, et al, 1992). This must definitely be done at lower taxonomic levels (classifiers), where there is the opportunity of better concretization and definition. The most general anthropogenic influence is manifested in two directions, as follows: - first – changes of natural soils under the influence of different factors and conditions, caused by man, within which the genetic (horizon) composition of the soil profile is preserved, but gradually modern processes start running. They alter the soil properties and thus formation of the anthropogenic horizon is virtually observed; second – appearance of new soils by natural or anthropogenic influences from different (mixed) soil-formation materials, most often of soil or rock origin. In this case the profile has a layered (binomial) structure, which is characterized by a different origin, composition and content. Nevertheless, in both cases the changes are caused directly or indirectly by man and he is the main, basic and primary factor of the line of the anthropogenic soil-formation process. The different agricultural methods, ways and forms of anthropogenic impacts determine the occurrence of different processes, which form very specific in composition, properties and structure soils

1. Building paddy fields, secondary water logging (glaying) processes and the formation of the so called ”rice soils”, which are spread in the Upper Thracian valley (Plovdiv and Pazardzhik region). Old abandoned paddy fields are still observed in the regions of Petrich, Haskovo, Yambol and other southern regions as well as the lower watercourses of the Maritsa, Struma and Mesta rivers on heavy, clayey and hydro-morphed soils (Teoharov, Ninov, 2005) These soils are initially formed in a mechanic way, the surface horizon of the zonal soils serving for enclosure walls of the paddy fields, in some regions called “smelters” because of the water logging during the rice vegetation.

The water layer according to the FAO classification is determined as anthraquic, and the one below as hydragric. According to their essence the newly formed soils are characterized by seasonally (spring-summer) caused water logging and must be determined as secondary seasonally water-logged soils. The soils initially acquire a new structure of the B_1–B_2–C, type and later – the $B_{1(g)}$–$B_{2(g)}$ because of the continuous underwater position during the vegetation period and the running of anaerobic and gleying processes. They are characterized by hydro-morphed stages of development with the occurrence of grayish spots in the wet period and rusty-black ones in the dry period which is a proof of acidification processes, the formation of sesquioxides and the occurrence of oxidation and reduction processes of iron and manganese taking active part in them. They are characterized by a hydro-morphed stage of development with appearance of grayish spots in the moist period and rusty-black ones in the dry one, which are a proof of acidification processes, sesquioxide formation and occurrence of acidification-reduction processes with the active participation of iron and manganese.

2. Terrain terracing surface horizon excavation and the formation of the so called "excavated soils", which are spread over sloping and hilly-ridge areas, where the terrain is leveled and terraced for forestry and agricultural purposes or for road construction, railway

lines, etc. The soils acquire a new structure of the B1–B2–C–D type. According to research by Myanushev and Teoharov (1990), these soils are determined as soils of artificially disturbed (eroded) profile. The authors artificially eroded the profile by excavating layers of up to 60 cm of A and B horizons of leached Leptosols and Calcic Chernozem, i.e slight, average and strong excavation (eroding) by mechanical withdrawal is applied. In their essence these are artificially eroded (excavated) soils of almost all degrees of erosion. Traditional tillage and mineral fertilization for growing agricultural crops have been applied. The soil properties have been studied for a period of 8 years. It has been established that for the period of research there have been no durable alterations of the chemical and physico-chemical properties of the modeled soils. According to the FAO classification these soils are determined on second level as escalated, i.e. terraced soils.

3. Trenching and mixture of horizons determine soils as "trenched " and "aerated" known also as soil ripping and subsoiling. These soils have wider distribution on terrains of vine and orchard plantations. The aim is to create suitable conditions for their root system. During the aeration, which is done until and about depth of up to and around 60 cm substantial alterations occur in the mixed A and B horizons, which lose their natural horizon; profile structure, which acquires a new habitué of new BA, AB, AC, CA horizons, i.e. they are mixed, splintered or inverted. The new (modified) soil changes its morphology, color, structure, contents and properties (chemical, physical, water-physical, physico-chemical, microbiological and biological). The altered characteristics of the particular indicators depend on the degree of mixing of the separate (A and B) horizons. It is very often that the soil profile turns from differentiated into undifferentiated and it inherits the properties of loose (soft) soil-formation rocks (in Regosols, Solonchack, Solonets, Chernozems, Rendzinas) or of the clayey alluvial horizons (Podsolic and Pseudopodsolic). At aeration of Pseudopodsolic soils at depth of 40-50 cm, Ninov and Teoharov (1986) determine alteration the morphological properties, the amount of soil moisture, the mobile forms of iron, aluminum and manganese, thus the soils are correctly named Aric Anthrosols. The term “trenching” is incorrectly introduced into science and practice meaning “trench”"or “ditch" originating from the French word “rigole”. A suitable classificatory for these soils is “fractic”.

4. Deposition of alluvial soil materials and overlapping of naturally formed soils. This process leads to formation of the so called Colluvisols (Teoharov, Ninov, 2005). They are distributed in different regions of the country and occupy the lower (accumulative) parts of the relief. Most frequently the alluvia overlap the zone Cambisols and Luvisols at the foot of the mountains - the brown soils at the lower part of the mountain, the arable ameliorated ones - at the depression parts of the fields. The overlapping of natural soils is also implemented through the spread of large amount of soil mass during excavation activities in construction (non-industrial) sites, open irrigation canals, irrigation systems (reservoirs and dams), etc. The main and basic factors for the deposition of alluvial soil materials are the mankind (root cause) and water. Logging and burn of forests are the exact reason for torrents, floods and modern soil formation of layered structure above the old soil formations. Teoharov et al., (1995) have conducted research on soils at the foot of southwest Pirin mountain and have found out diluvia soils of complex two-membered (modern) and relict (on former Leptosols) profile of the following structure: ACк I layer–ACк II layer–BCK III laye.-Ck IV layer–AB$_1$k–B$_2$tk–Ck.

The profile indicates that initially there were erosion processes (A horizon not available) and the alluvial layers were formed thereafter. Similar soils were studied by Duchaufour (1988) at the foot of Jura Mountain (France) and he defined them as poligenetic. . According to second level of FAO classification they are determined as terric.

5. Drainage of hydromorphic soils and their transmission into automorphic.

Drainage is performed in regions of marshy soils such as Gleysols, Histosols, Solonchak, Solonetz, etc. Thus the soils of hydromorphic stage of development pass into automorphic and due to the change in their water status they are called "drained" or "hydro meliorated". According to the hydro geological, soil and other conditions the ameliorations are conducted by a surface (open), subsoil (closed), horizontal or vertical drainage (Kavardziev, 1985).

The drainage systems applied regulate the level of subterranean waters by limitation of the water outflow and elimination of the inflow of nurturing waters. They lead to full water repellency of soil and the level of the water layer is substantially below critical. Processes of dehydration and oxygenation of soil, activation of microbiological processes as well as transformation of organic and mineral substances into mature humus and free chemical compositions and plant absorbent nutrients occur.

Processes of hydromorphic sod formation, sod availability, turf formation, over wetting and claying are completely reduced due to the permanent drainage of the soil profile and can be used for growing agricultural crops. Thus the diagnostic hydraquic, hydragric и histic horizons alter their properties and characteristics and the newly formed horizons and soils, according to FAO criteria are determined as drainic. From ecological point of view the hydromorphic (marshy) soils should not be drained as that would disturb the habitat of the fauna in the whole environmental niche.

6. Excessive fertilization of garden soils with organic manure (muck, compost, etc.), use of non-regulated manure deposits and water contamination.

These soils have been formed due to continuous planting of vegetable and flower crops in yards and greenhouses even due to an exceeding desire of conducting organic agriculture. Imposing agrochemical and agroecological control is mandatory especially in regions of water deficiency (in Dobrudza area) or regions of nearby subterranean water around river arteries, where in both cases well water is used for household purposes. The main disadvantage of soil use in these regions is that in yards manure deposits with protective (concrete) walls for manure storage are not constructed an it is dissiminated into gardens and greenhouses before undergoing the necessary microbiochemical fermentation. The annual organic fertilization combined with frequent watering of the crops planted leads to contamination of well water with organic acids, salts and different chemical elements which manure is abundant of. Nowadays producers use non-decomposed poultry manure, which leads to water contamination substantially above the maximum admittable concentrations. The excessive enrichment of garden soils with organic nutrients is not a good indicator of their qualities and the agricultural products as well as to the health of soil and man. Agrochemical and agroecological control should be additionally conducted on the basis of lyzimetric research of waters and soil. Otherwise garden type of soils of hortic horizon and good agroecological condition are transformed into organically overloaded and contaminated ones having acquired plaggic horizon and degraded balance.

7. Soil contamination from plant protection and mineral fertilization.

The annual conduction of plant protection for preservation of plants of diseases and pests and their nourishment with mineral fertilization lead to the accumulation of residues (contaminators) in soils, agricultural products and people. Insufficient conservation protection and guarding of plant protection preparations left in storehouses and sites after the system change in agriculture have reflected in a negative way the local anthropogenic and agrochemical soil load. Copper accumulation in the surface horizons of massive fields of vines, orchards, cereals and vegetable crops due to the use of copper preparations and substantial qualities of nitrogen and nitrogen compounds in the lower horizons and waters have been established and it is due to unbalanced application of nitrogen and acidic fertilizers.

The soil horizons are defined as anthrotoxic because of the genesis of their formation. Te strict observation of Bulgarian State Standard of preparations and the nitrite directive are not sufficient for extinction of these processes. The application of lyzimetric methods for establishing of the soil contamination is of specific importance.

Classification:

1. Secondarily superficially wetted - Anthraquic-Hydragric – characterized by a excavated for ridges of rice fields surface horizon and a newly formed seasonally over wetted anthraquic хоризонт with depth of ≥ 15 cm и hydragric horizon of depth ≥ 10cm, gleying processes - indicators of a glayic хоризонт with yellowish-brown, reddish-brown and iron-manganese spots.
2. Artificially eroded - Escalic – they have an excavated surface horizon and a newly formed one turned into a surface one of transient of AB, (B), Bt или AC characteristics of the natural soil with qualifier anthropogenic terrace (escalic) и anthric properties.
3. Aerated - deeply ploughed (Fractic) – they have an altered horizontal profile, anthric properties and fractic qualifier, deepening up to 40-50 (60) cm by roll over, mixture and breaking of soil and degradation of its initial structure.
4. Colluvisols - buried (Terric) - they have a alluvia layer of soil with a depth of ≥ 5 до ≤ 50 cm and formation of a terric horizon, covering the original (buried soils).
5. Hydromeliorated - drainedи (Drainic) – they have a drained hydromorphic soil profile, turned into an automorphic one through drains. Their qualifier is drainic soils.
6. Organic - garden (Hortic) - with a hortic horizon, depth of 20 cm, organic carbon ≥ 1,0 %, conditions for fast turfing with acid grass varieties.
7. Anthrotoxic - contaminated - with strongly anthric properties and a qualifier anthrotoxic. They contain toxic (above maximally admitted concentration) substances of mineral fertilizers and plant protection preparations.

References:

Duchaufour, Ph., 1988. Pedologie, Masson, Paris, 224 p.

FAO. 2014. World Reference Base for Soil Resources., Rome, 151p.

Kavardziev Y., 1985, Salinized Soils. Amelioration of Soils of Unfavorable Properties, Zemizdat, 117-240 pp, (in Bulgarian).

Myanushev E., M. Teoharov, 1990. Condition of Some Physico-chemical Properties of Soils of Artificially Disturbed Profile; Soil Science and Agro-chemistry Journal, issue № 5-6, 3-11 pp., (in Bulgarian).

Ninov N., M. Teoharov, 1986. Contribution to the Classification and Diagnostics of Podsolic (Pseudopodsolic) soils, Affected by Anthropogenic Influences; IVth National Conference of Soil Science; Bulgarian Soil Science Society, Sofia, 42-47 pp, (in Bulgarian).

Penkov M., V. D. Donov, T. Boyadjiev, T. Andonov, N. Ninov, M. Yolevski, G. Andonov, S. Gencheva. 1992. Classification and Diagnostic of Bulgarian soils Related With Land Reform, Zemizdat, Sofia, pp. 151, (Bulgarian) .

Teoharov M., N. Ninov, 2005. Sustainable Development of Agriculture in the Region of Petrich, Monography, PSSE, 144 p, (in Bulgarian).

Teoharov M. 2019. Anthrosols, In: Genetic and Applied Classification of Soils and Lands in Bulgaria. Ed. M. Teoharov. BSSS, 140-147, Sofia (in Bulgarian).

Treykyashki P., B. Hristov, I. Babev, 1982. Grouping of Geological Materials According to Degree of Suitability for Biological Reclamation; Digest of III-rd National Conference of Soil Science, part I, 87-90 pp, (in Bulgarian).

Influence of the Red Wood Ant *Formica lugubris* Zetterstedt, (Hymenoptera: Formicidae) on the Surrounding Forest Soil

Angelos Tsikas*, Paraskevi Karanikola*,
Michail Orfanoudakis**
Department of Forestry and Management of the Environment and Natural Resources, Laboratory of Forest Protection, Democritus University of Thrace, School of Agricultural and Forestry Sciences, Ath. Pantazidou 193, 68 200 Orestiada, Greece
***Department of Forestry and Management of the Environment and Natural Resources, Laboratory of Soil Science, Democritus University of Thrace, School of Agricultural and Forestry Sciences, Ath. Pantazidou 193, 68 200 Orestiada, Greece*
Corresponding Author: Angelos Tsikas, e-mail: atsikas@fmenr.duth.gr

Received: 12 October 2020 *Accepted: 10 March 2021*

Abstract

Red wood ants build their nests as above-ground mounds by collecting organic materials from the forest floor. The effects of ant nest to the soil carbon and nitrogen along with the soil pH, were measured. There was an increase in pH with distance from the nests while there was a decrease in C and N concentration with the distance from the nest increases. The carbon content at the mound and nests center was differ, with carbon percentage more than double at the nests mound than in the center of the nest. In contrast the nitrogen percentage was higher at the nest center but with no significant difference. The pH was increased at the nests center while the soil was more acidic at greater ratio. Red wood ants' mounds do not significantly alter the C and N concentrations in the forest ecosystem, but they increase spatial heterogeneity of the forest floor by storing organic matter and nutrients.

Keywords: Red wood ants, pH, C and N concentrations, forest soil, forest ecosystem.

Introduction

Ant nests are considered as natural occurring disturbances in the forest ecosystem, contributing to the soil property complexity as it was described by Wagner et al. (1997). Red wood ants, (particularly the members of the *Formica rufa* Group) could be found mostly in coniferous forests as well as in mixed and deciduous forests of the temperate zone. Their nest is made of organic materials from the forest floor, they are above ground and can remain active for many years (Holldobler and Wilson, 1990).

The chemical properties and decomposition rates of the nest building material differ from those of the forest floor. Several factors, like low moisture, higher average temperature, and chemical properties along with microbial activity influence decomposition rate in the nest (Czerwiński et al., 1971; Pokarzhevskij, 1981; Frouz, 2000). By that, the carbon pool in the nest is higher than in the forest floor and soil (Frouz et al., 1997; Laakso and Setälä, 1998; Lenoir et al., 2001). Lenoir et al. (2001) in Swedish scots pine forests, found that C concentrations can be nearly 20% higher in the center of the mound than in the forest floor, while N concentrations differed from site to site. The C and N concentrations were always higher in four different forest types in Switzerland, even though C concentrations were significantly higher among forest types whilst N concentrations were not (Risch et al., 2005). Frouz et al. (1997) found similar results in the Czech Republic. Such carbon pools however contain only a small fraction of the total forest floor carbon pool, but they contribute significantly at the spatial heterogeneity of C distribution in the forest ecosystem (Rosengren and Sundstrom, 1987; Gosswald, 1989).

Ant nests are well known also for altering soil pH significantly, tending to neutralize it (Dlusskij, 1967; Frouz et al., 2003). In acidic forest soils, ant nests have higher pH than humus (Brady and Weil, 1999) and forest soil (Jilkova et al., 2010). Laakso and Setälä (1997) in Finnish boreal forests found that pH on the nest surface can be significantly higher than that of the soil. Though, not all ant species alter the pH in the same manner e.g., *Formica polyctena* Förster, 1850 and *Formica lugubris* Zetterstedt, 1938 alter pH from acid to neutral values more intensively than *Formica pratensis* Retzius, 1783 (Malozemova and Koruma, 1973).

Even though there are many studies on the impact of red wood ants in soil in even-aged boreal and central Europe forests, studies in Mediterranean or all-aged forest stands are lacking. In the present study we measured pH and C and N concentrations in the red wood ants' nests and the surrounding soil. The purpose of this research was to find the differences of these factors between the nests and the soil in an all-aged forest.

Materials and Methods

The study area was in Elatia forest, located in Northern Greece (41° 28 '50.85' N, 24° 19'16.70' E) with an area of approximately 7 hectares (7,034 ha). The altitude ranges between 1500-1650 meters with mild to moderately strong slope. It is an all-aged managed forest dominated by Norway spruce (*Picea abies* L. H. Karst), Scots pine (*Pinus sylvestris* L.), and Copper beech (*Fagus sylvatica* L.). The site type is classified as Vaccinio-Piceion (Cajander, 1949). According to the closest meteorological station in Skaloti (altitude 900m) the average temperature is 11.46 ^{o}C and the average annual precipitation is 955.1 mm, However, at the study area precipitation was higher, as it was reported by Ganatsas (1993).Bedrock of the study area is dominated of granite and gneiss (Zagas, 1990; Tsiaoussi, 1996), while at the location of the recorded nests, the parent rock material was gneiss.

In early August 2013, a total of 46 nest mounds were counted in the study area; five typical representative mounds were selected. Stockan et al. (2016) key was used to identify the species. A soil sample of 0-20cm and 10-30cm soil depth was sampled at points 0,5m and 1m uphill and downhill from the ant mound edge, 0-20cm soil depth from the center of the nest, and nest building materials from the center of the above ground nest with a soil corer and trowel, a total of 55 soil samples. The samples were oven dried, sieved through 0.8cm and 0.2cm sieve and stored in a polyethylene (PE) bag.

In the laboratory, we measured pH at 1:1 soil water suspension (Thomas, 1996). Organic matter (OM) and carbon (C) were measured by the Walkley and Black method (1937), and total nitrogen (N) was determined with the Kjeldahl digestion (Bremner and Mulvaney, 1982).

Data statistical analysis was made with one way ANOVA followed by a Tukey posthoc test for pairwise comparison, while the N data did not follow the normal distribution, and therefore they analysed with non-parametric Kruskal Wallis test, followed by U Mann-Whitney tests.

Results and Discussion

All nests belonged to the 'hairy red wood ant' (*Formica lugubris* Zetterstedt, 1938).

Soil pH seems to decrease as we fend off the mound (Fig. 1). The mean pH values are from 4.84 at the 1 m distance to 4.94 and 5.08 at the center of the nest (Fig. 2, Table 1). PH neutralization in the center of the nest is well documented in several other studies. The species *Acrogypa sp.* in Bode et al. (1999) in acidic soils in Iowa, as well as *Formica perpilosa* Wheeler, 1910 in arid regions of south-western United States (Wagner et al., 1997) also tended to neutralize the pH in their nests. The soil layer near the nest was also affected showing an increased pH. However, soil acidity may not be influenced by the accumulated litter at the nest. Coniferous forests' litter has the tendency to acidify the soil layers below. Such pattern was not present at the present or any other similar studies. Suggesting no direct relation among the litter and humus layers to the soil chemistry at the nest region. Other microbiological related factors could explain these results.

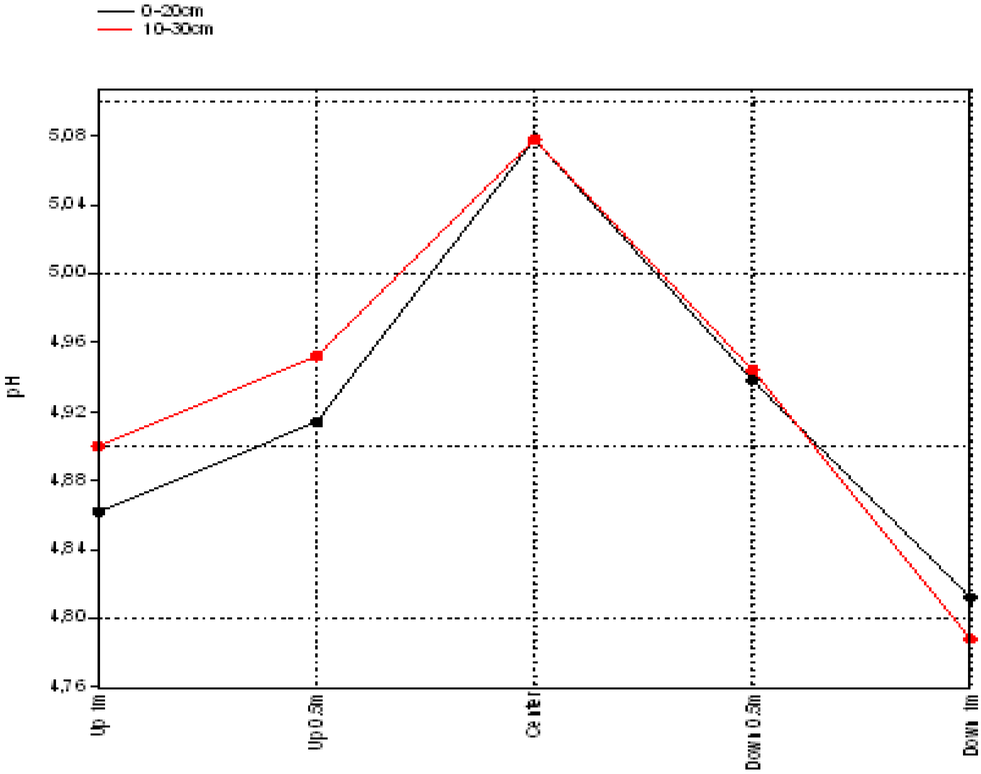


Fig 1. *Changes in pH value uphill (Up), in the center (Center) and downhill the nest (Down).*

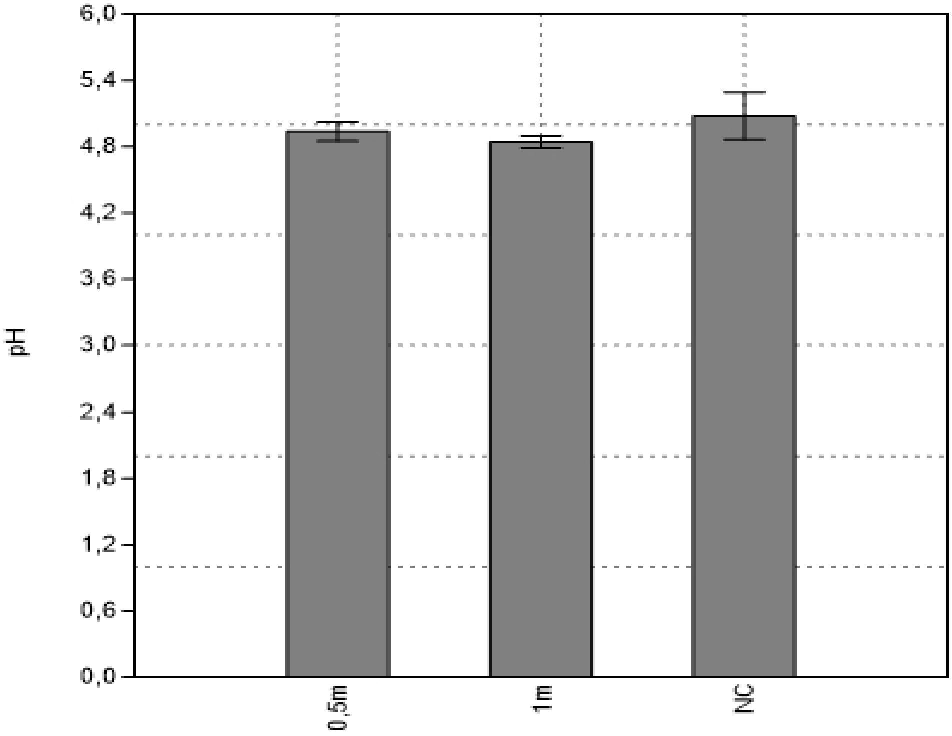


Fig 2. *Mean pH and standard error of soil from distances of 0,5m, 1m and from the center of the nest (NC).*

Table 1. *pH values in 0.5m and 1m distance from the mound, and in the nest center.*

	pH		
	0.5m	1m	nest center
Max.	4.340	4.320	4.540
Min.	5.820	5.400	5.600
Mean	**4.937**	**4.841**	**5.078**
SD	0.384	0.244	0.478

Both nitrogen (Fig. 3, 4) and carbon (Fig. 5, 6) were significantly higher in above-ground mounds than the average values in reference soils (Table 2). That was as expected since the nest building material was consisted mainly of coniferous needles. The data analysis of the C and N content agree with the existed literature, as it was documented in coniferous forests in Moscow (Zakharov et al., 1981), in subalpine coniferous forests in Switzerland (Risch et al., 2005) and in mixed coniferous-broadleaf forests in Finland (Kilpeläinen et al., 2007). The hypothesis that the accumulated litter may have significant effect on the chemical properties probably is not correct. The main building material of the above-ground mounds consists mainly of coniferous needles. This explains the high concentrations of C. In contrast, N concentrations in the nest is much higher that the litter. Increased microbial activity (Lenoir et al., 2001), as well as the increased resin content of the building material of the nests explain the findings. Similar results were described by Berg et al. (2000). Frouz et al. (1997) found a much higher number of nitrogen-binding bacteria in the nests of red wood ant *Formica polyctena* Foerster, 1850 compared to the forest floor.

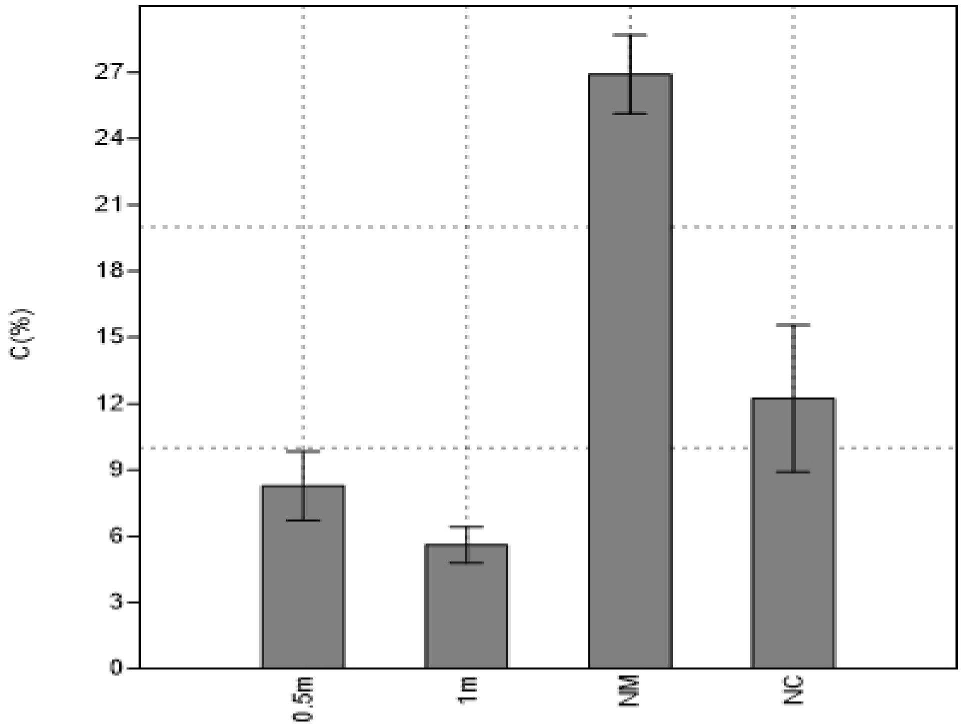


Fig 3. *Mean C concentration (%) and standard error of soil from distances of 0,5m, 1m, from the mound material (NM) and from the center of the nest (NC).*

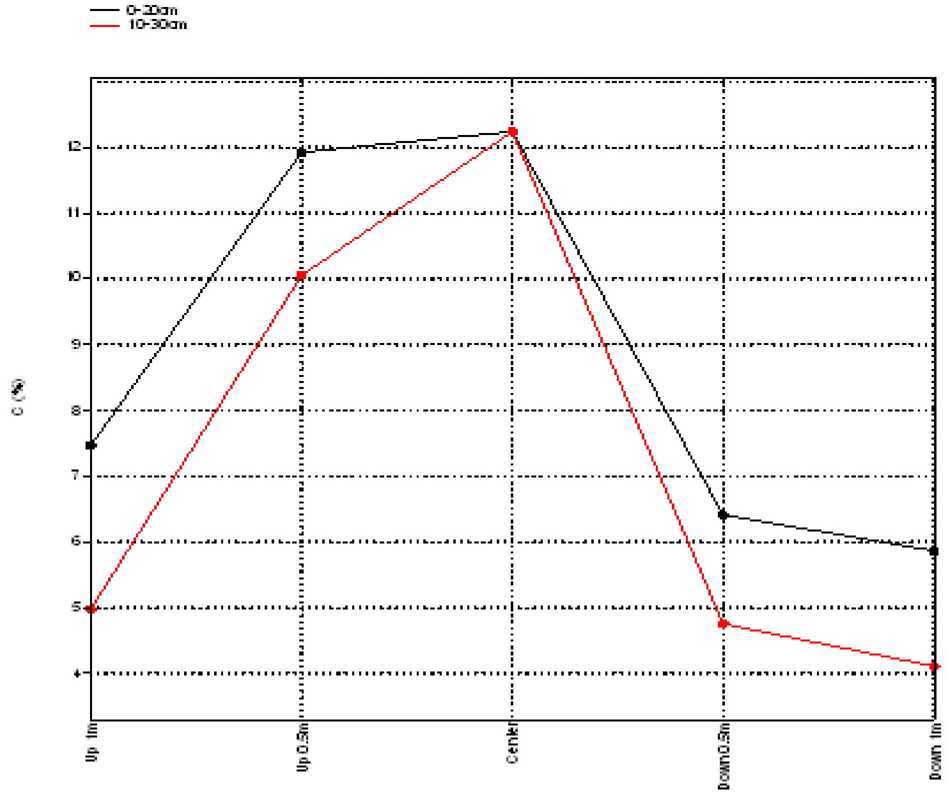


Fig 4. *Changes in C concentration (%) uphill (Up), in the center (Center) and downhill the nest (Down).*

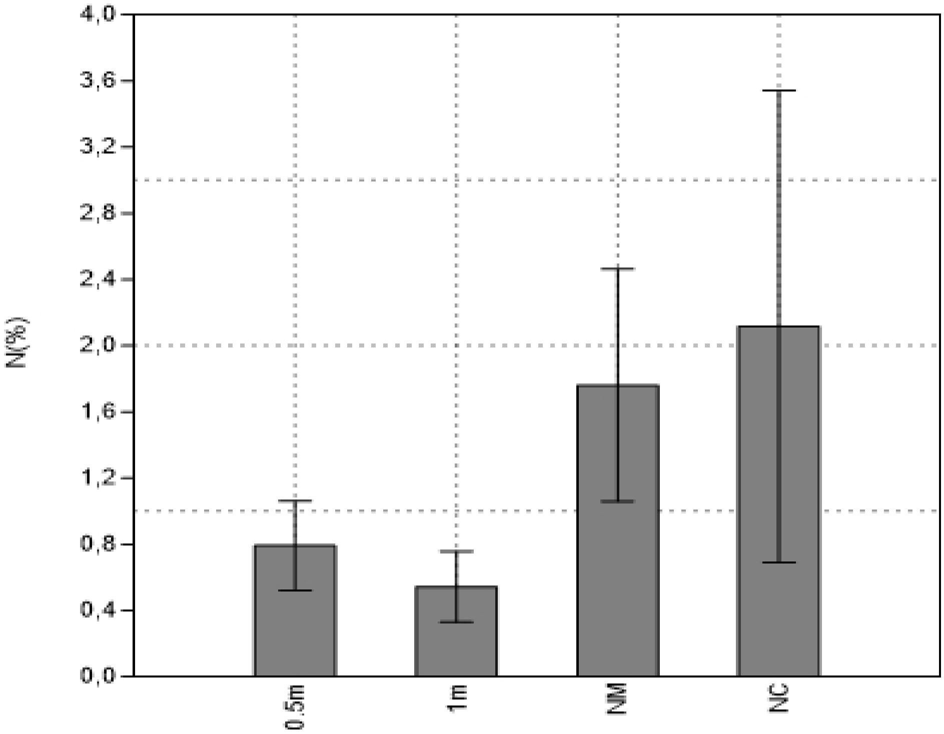


Fig 5. *Mean N concentration (%) and standard error of soil from distances of 0,5m, 1m, from the mound material (NM) and from the center of the nest (NC).*

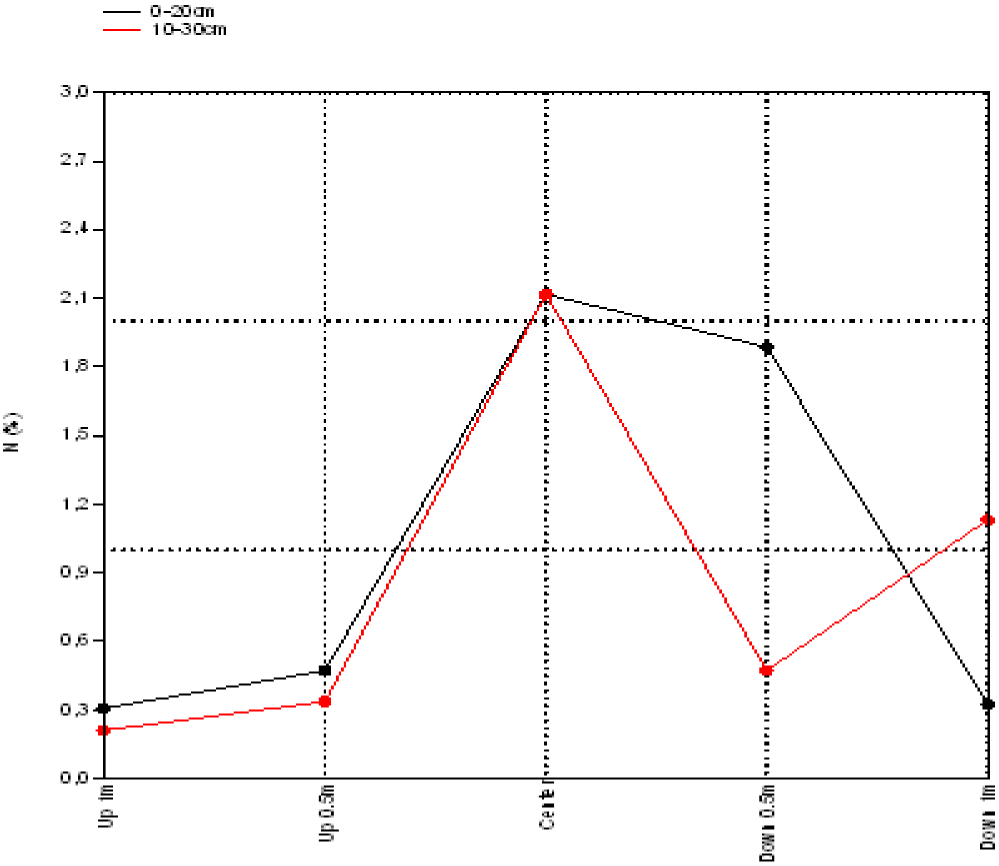


Fig 6. *Changes in N concentration (%) uphill (Up), in the centre (Center) and downhill the nest (Down).*

Table 2. *C and N concentrations (%) in 0.5m and 1m distance from the mound, in the nest building materials and in the nest center.*

	0.5m		1m		Nest material		Nest center	
	C	**N**	**C**	**N**	**C**	**N**	**C**	**N**
Max.	0.706	0.054	0.319	0.083	17.787	0.802	4.027	0.147
Min.	25.157	5.201	12.482	4.464	34.557	8.048	24.160	7.710
Mean	**8.287**	**0.792**	**5.605**	**0.543**	**26.914**	**1.761**	**12.240**	**2.117**
SD	6.969	1.204	3.668	0.957	5.633	2.217	7.409	3.188

Conclusion

Red wood ants increase pH at the nest in acidic soils, although such effect is minimal. Their influence on the forest soils is minimal, although they significantly increase the heterogeneity of the soil environment. They do not seem to alter the chemical properties of the soil significantly, but they increase the spatial heterogeneity of the forest soil in organic matter and nutrients. These are stored and retained in the nests, and when the colony is abandoned, they decompose and return to the soil, contributing to geochemists cycles locally.

References

Berg, B., M.B. Johansson, V. Meentemeyer, 2000. Litter decomposition in a transect of Norway spruce forests: substrate quality and climate control. Can. J. Forest Res. 30, 1136–1147.

Bode, S., A. Goose, C. Warpehoski, 1999. Ant mounds influence soil composition, but not vegetation. Tillers, 1, 25-29.

Brady, N.C., R.R. Weil, 1999. The Nature and Properties of Soils. Prentice-Hall: Upper Saddle River, New Jersey.

Bremner J.M., C.S. Mulvaney, 1982. Nitrogen total. In: Methods of soil analysis. Agron. no. 9. Part 2: chemical and microbiological properties, 2nd edn, Page AL (ed). American Society of Agronomy: Madison, WZ; 595–624.

Cajander, A.K., 1949. Forest types and their significance. In: Acta Forestalia Fennica 56(5): 1–71. Helsinki: Finnish Society of Forest Research.

Czerwiński Z., H. Jakubczyk, J. Pętal, 1971. Influence of ant hills on the meadow soils. Pedobiologia, 11, 277–285.

Dlusskij, G.M., 1967. Muravji roda *Formica*. Nauka, Moskva 236pp. (in Russian)

Frouz, J., H. Santruckova, J. Kalcik, 1997. The effect of wood ants (*Formica polyctena* Foerst.) on the transformation of phosphorus in a spruce plantation. Pedobiologia, 41, 437–447.

Frouz, J., 2000. The effect of nest moisture on daily temperature regime in the nest of *Formica polyctena* wood ants. Insectes Sociaux, 47, 229-235.

Frouz, J., M. Holec, J. Kalcik, 2003. The effect of *Lasius niger* (Hymenoptera, Formicidae) ant nests on selected soil chemical properties. Pedobiologia, 47, 205-212.

Ganatsas, P., 1993. Stand structure and natural regeneration of spruce forest in Elatia Drama, northern Greece. Ph.D. Thesis. School of Forestry and Natural Environment, Aristotle University of Thessaloniki, Greece.

Gösswald, K., 1989. Die Waldameise. Band 1. Biologische Grundlagen, Okologie und Verhalten. AULA, Wiesbaden, Germany. (in German)

Hölldobler, B., E.O. Wilson, 1990. The ants. Harvard, Cambridge, Massachusetts, USA.

Jilkova, V., J. Frouz, T. Domisch, L. Finer, 2010. The effect of wood ants (*Formica s. str.*) on soil chemical and microbiological properties. 19th World Congress of Soil Science, Soil Solutions for a Changing World 1 – 6 August 2010, Brisbane, Australia. Published on DVD.

Kilpeläinen, J., L. Finer, P. Niemela, T. Domisch, S. Neuvonen, M. Ohashi, A.C. Risch, L. Sundström, 2007. Carbon, Nitrogen and Phosphorus dynamics of ant mounds (Formica rufa group) in managed boreal forests of different successional stages. Applied Soil Ecology 36:156–163.

Laakso, J., H. Setälä, 1997. Nest mounds of red wood ants (*Formica aquilonia*): hot spots for litter-dwelling earthworms. Oecologia, 111, 565-569.

Laakso, J., H. Setälä, 1998. Composition and trophic structure of detrital foodweb in ant nest mounds of *Formica aquilonia* and in the surrounding forest soil. Oikos, 81, 266–278.

Lenoir, L., T. Persson, J. Bengtsson, 2001. Wood ant nests as potential hot spots for carbon and nitrogen mineralization. Biology and Fertility of Soils, 34, 235–240.

Malozemova, L.A., N.P. Koruma, 1973. Influence of ants on soil. Ekologia, 4, 98-101. (in Russian)

Pokarzhevskij, A.D., 1981. The distribution and accumulation of nutrients in nests of ant *Formica polyctena* (Hymenoptera, Formicidae). Pedobiologia, 21, 117–124.

Risch, A.C., M.F. Jórgensen, M. Schótz, D.S. Page-Dumroese, 2005. The contribution of red wood ants to soil C and N pools and to CO2 emissions in subalpine forests. Ecology, 86, 419–430.

Rosengren, R., L. Sundstrom,1991. The interaction between red wood ants, *Cinara* aphids and pines. A ghost of mutualism past? In: Huxley, C.R. & Cutler, D.F. (Eds.), Ant-plant interactions. Oxford University Press, Oxford, New York, Tokyo, pp. 80–91.

Stockan, J.A., E.J.H. Robinson, J.C. Trager, I. Yao, B. Seifert, 2016. Introducing wood ants: evolution, phylogeny, identification and distribution. In: Wood Ant Ecology and Conservation, eds. Jenni A. Stockan and Elva J. H. Robinson. Published by Cambridge University Press. Cambridge University Press 2016 pp. 17-52.

Thomas, G.W., 1996. Soil pH and soil acidity. p. 475–490. In D.L. Sparks (ed.) Methods of soil analysis. Part 3. Chemical methods. SSSA Book Ser. 5. SSSA, Madison, WI.

Tsiaoussi, V. (Project Leader), 1996. Specific Management Plan for the Site Periochi Elatia (GR1 140003). The Goulandris Natural History Museum-Greek Biotope/Wetland Centre. Thermi. 197 p.

Wagner, D., M.J. Brown, D.M. Gordon, 1997. Harvester ant nests, soil biota and soil chemistry. Oecologia, 112, 232-236.

Walkley, A., I.A. Black, 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science, 37(1), 29-38.

Zagas, T., 1990. Conditions for natural establishment of Pinus sylvestris in an area of Rhodopi mountain, northern Greece (p. 170). Ph.D. thesis. Thessaloniki: Aristotle University.

Zakharov, A.A., E.F. Ivanickaja, A.E. Maksimova. 1981. Accumulation of nutrients in nests of *Formica sp*. (Hymenoptera, Formicidae). Pedobiologia 21, 36–45.

Ionic Mobility of Cations as Affected by Redox Status of Two Different Soil Textures

G.O. Dayo-Olagbende and B.S Ewulo

Department of Crop Soil and Pest Management, Federal University of Technology Akure,
PMB 704, Ondo State Nigeria

Corresponding Author: G.O. Dayo-Olagbende, e-mail: phemmieisrafel@gmail.com

Received: 5 January 2021 *Accepted: 25 March 2021*

Abstract

Movement of ions within the soil plays a key role in nutrient availability as well as soil management. Various reactions in the soil contribute to soil ionic mobility and consequently nutrient availability, one of which is the oxidation-reduction reaction. Hence, this study sets out to evaluate the effect of redox status of the soil on ionic mobility of cations. A 3x2x2 factorial experiment was set up on two different soil types (clay loam and sandy clay loam), arranged in a Randomzed complete Block design (RCBD) with three replicates. The treatment combination involved three levels of poultry manure (0 tha^{-1}, 6 tha^{-1} and 8 tha^{-1}), two levels of NPK 15-15-15 (0 gha^{-1}, 200 kgha^{-1}) and two water regimes (field capacity and waterlogging). Twelve treatments were obtained giving rise to different redox potential. Redox potential was measured using a redox meter, electrical conductivity using a conductivity meter; Ionic mobility was calculated using the mobility equation.

Results from this study show that ionic mobility was highest under highly reduced conditions, also ionic mobility was highest in sandy clay loam than clayey loam and potassium ion has the highest mobility while magnesium has the lowest mobility. It was concluded that soils can be subjected to reduction process to increase the mobility of cations hence there availability to plant. However care must be taken to avoid the loss of highly mobile ions like potassium.

Keywords: ionic mobility, redox, cation, soil, nutrient availability, movement

Introduction

Essential mineral nutrient elements are taken into the plant system by the roots and they exist in soil solution as ions (Singh and Schulze, 2015). These elements can only be used by plants in their ionic forms. Cation and anion absorption by the plant root is determined by both root physiology and the movement of ions in the soil itself and within the soil solution. The nature of the mobility of these ions in the soil solution affects plant growth, crop yield, and product quality (Ehsan et al., 2010). Mobility of ions could be in form of mass flow, diffusion as well as root interception, these processes determine the availability of these ions for plant uptake. Anions and cations in solution are moved mostly by diffusion, and it involves the movement of ions within water that exist around soil particles, the driving force being the ion concentration gradient, always moving from an area with higher concentration to an area of lower concentration (Drew et al., 1969). Redox reactions involve the transfer of electron from one compound to another. This type of reaction involves an oxidant and a reductant, the oxidant is the electron acceptor and in most cases, is the soil components while the reductant is the electron donor which includes various soil additives. Over the years, pH has been considered as the major soil reaction (Brady and Weil, 2010; Simek and Cooper, 2002), however in recent days, the concept of redox potential or electron flux is gaining attention as a soil chemical reaction. The redox process affects many biogeochemical processes of anaerobic systems like lakes, waterlogged soils, sediments and groundwater (Heron et al., 1994). It also affects the behavior of nutrient elements and is involved in the solubility and toxicity of heavy metals in the soil (Chuan et al., 1996) as well as the chemistry of living organisms (Dietz, 2003; Falkowski et al., 2008; Greenberg, 1998). Redox potential has been used in many disciplines that deal with living organisms, such as microbial ecology (Alexander, 1964), limnology (Reddy and DeLaune, 2008), bioenergetics (Guérin, 2004; Mathis, 1995), soil science (Chadwick and Chorover, 2001), physiology and ecophysiology (De Gara et al., 2010; Dessaux et al., 2009; Dietz, 2003; Foyer and Noctor, 2005; Lambers et al., 2008). The activities of soil microbes are dependent on the soil reaction and oxidation-reduction potential (Falkowski et al., 2008), it also has effect on the development of microorganisms. Bacterial growth is directly correlated to changes in redox potential (Kimbrough et al., 2006) while microbial and enzymatic activities are negatively correlated with redox potential in anaerobic soils (Brzezinska, 2004; Kralova et al., 1992). Furthermore, the redox state of nodules is regarded as a referee of legume-rhizobium symbiosis (Marino et al., 2009) and nitrogen fixation by Azospirillum spp. is governed by soil redox fluctuations (Charyulu and Rao, 1980). The source of electrons for biological reduced environment is organic matter (Oglesby, 1997; Chadwick and Chorover, 2001; Lovley et al., 1998). Redox potential and pH mainly influence the form under which N is assimilated by plants. In plant nutrition, the availability of P is greatly influenced by both redox potential and soil pH (Kemmou et al., 2006; Phillips, 1998; Sallade and Sims, 1997; Vadas and Sims, 1998).

Recently, researchers have emphasized using fertilizers from organic sources in agronomic practices (Timisina, 2018) or a combination of both organic and inorganic fertilizers to boost productivity and soil fertility (Roba, 2018). Organic manure from poultry sources has been found to improve the yield of maize and soil infiltration dynamics (Akingbola et al., 2017). Organic residues from tithonia have also been found to improve soil chemical properties and Okra yield (Amodu et al., 2019). This practice has a direct influence on the soil redox status and, consequently on ionic mobility. However, researches that have looked into soil redox potential have not given much information on its effect on movement of ions which plays a vital role in nutrient availability and plant nutrition. Hence, the objective of this research is to investigate the ionic mobility of cations as affected by the redox status of two different soil textures.

Materials and Methods

The site of the experiment was at Apatapiti layout around the west gate of the Federal University of Technology, Akure (FUTA) Ondo State Nigeria. It lies within the tropical rainforest belt in southwestern Nigeria within latitude 5° 16'N -5° 22'N and longitude 15° 11'E -15° 16'E.

The study was conducted using two soil types, the soils used are Alfisols according to USDA classification. They were formed under forest and have a subsurface horizon with clay accumulated, however, both fall under different textural classes according to the textural triangle. Site 1 is a sandy clay loam having a sand content of 54.8%, silt content of 20.5% and clay content of 24.7%. The soil at site 1 has an organic carbon content of 0.92%, total nitrogen of 0.19% and available phosphorus of 15.2 ppm. Site 2 is a clayey loam having a sand content of 48.0%, silt content of 21.4% and clay content of 30.6%. The soil has an organic carbon content of 1.37%, total nitrogen of 0.17% and available phosphorus of 28.8 ppm.

Experimental Design and treatments combination:

A 3x2x2 factorial experiment was set up on two different soil types (sandy clay loam and clay loam), arranged in a randomized complete block design (RCBD) and replicated three times. The treatment combinations involved three levels of poultry manure (0 tha^{-1}, 6 tha^{-1} and 8 tha^{-1}), two levels of NPK 15-15-15 (0 gha^{-1}, 200 kgha^{-1}) and two water regimes (field capacity and waterlogging). A total of twelve treatments were obtained, which gave rise to different redox potential they include; oxidized soils (Eh >300), moderately reduced soil (Eh range 200-300), reduced soil (Eh range -130 to -100) and highly reduced (Eh <-200).

Soil Analysis and Sampling:

Samples of the soil were randomly collected from the experimental site from 1 week after incubation (WAI) to 12 weeks after incubation (WAI) at an interval of 4 weeks (1WAI, 4WAI 8WAI and 12WAI). Samples collected were analyzed for redox potential and electrical conductivity from which ionic mobility was calculated. In analyzing for redox potential, a method similar to that described by Rabenhorst et al., 2009 was used, 20g of the soil samples were collected, soaked in water from bottom to top so as to prevent entrapment of air during saturation and allowed to mix for 30 minutes after which 50 ml of the solution was collected and taken to the laboratory for reading. In the laboratory, redox potential (Eh) was measured using a pH/Redox combined meter. Voltage was measured every 10 seconds for 60 seconds and the mean values of the collected measurements were calculated. The electrical conductivity was analyzed using the method of Rayment and Lyon (2011), 20g of soil was mixed with 50ml of distilled water and stirred at the interval for 30 minutes. The solution was then read with a conductivity meter. Particle size analysis was done to determine the textural classes of the soils used using hydrometer method. Ionic mobility was calculated using the electrical mobility equation.

$D_i = \frac{\mu KBT}{q}$, Making μ the subject of formula $\mu = \frac{Diq}{KBT}$

Where; D_i= Diffusion coefficient of ion, q= Electrical conductivity, K_B= Boltzmann's constant= 1.380649×10^{-23} J K^{-1}, T= Temperature and μ =Ionic mobility. Textural class analysis was done using the hydrometer method.

Statistical Analysis

Data obtained from this study were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) to obtain mean value and level of significance while graphs were generate using Microsoft excel 2010 edition so as to show the trends of findings.

Results

Table 1 presents the treatment combinations, their respective redox potential and their corresponding electrical conductivities; it also shows their respective oxidation-reduction status. T_1, T_3, and T7 are oxidized soils, T_5, T_9 and T_{11} are moderately reduced soils, (T_2 and T_4) are reduced soils and T_6, T_8, T_{10} and T_{12} are highly reduced soils. T_{12} has the highest electrical conductivity for both soils while T_1 has the lowest.

Table 1. *Classification of Treatment Based on Eh Range and their corresponding Ec*

Treatments	Treatment Description	Eh Range	Eh Class	Ec (Soil 1)	Ec (Soil 2)
T_1	0 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	200 to 370	Oxidized	480l	385k
T_2	0 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-120 to -100	Reduced	707j	473j
T_3	0 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	260 to 430	Oxidized	582k	732h
T_4	0 t ha^{-1} PM + 200 kgha^{-1} NPK + WL	-130 to -100	Reduced	815i	715i
T_5	6 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	200 to 290	Moderately reduced	913h	787g
T_6	6 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-240 to -210	Highly Reduced	1395f	1200cd
T_7	6 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	280 to 310	Oxidized	1260g	1005f
T_8	6 t ha^{-1} PM +200 kg ha^{-1} NPK + WL	-230 to -200	Highly Reduced	1646b	1215c
T_9	8 t ha^{-1} PM + 0 kg ha^{-1} NPK + FC	230 to 300	Moderately Reduced	1407e	1104e
T_{10}	8 t ha^{-1} PM + 0 kg ha^{-1} NPK + WL	-240 to -230	Highly Reduced	1642c	1500b
T_{11}	8 t ha^{-1} PM + 200 kg ha^{-1} NPK + FC	230 to 300	Moderately reduced	1426d	1182d
T_{12}	8 t ha^{-1} PM + 200 kg ha^{-1} NPK+ WL	-240 to -230	Highly Reduced	1740a	1605a

PM = Poultry Manure, NPK = NPK 15-15-15, WL= Waterlogged, FC= Field Capacity.

Figures 1 to 4 presents the effect of redox potential on ionic mobility of soil cations over a period of 12 weeks. Oxidized soils (T_1, T_3, and T7) are represented with blue lines, moderately reduced soils (T_5, T_9 and T_{11}) by green lines, reduced soils (T_2 and T_4) by red lines and highly reduced soils (T_6, T_8, T_{10} and T_{12}) by black lines. Similar trend was observed for all the cations throughout the trial period and on the two soil types. Highly reduced soils had the highest values throughout the trial period, followed by moderately reduced soils, oxidized soil and then reduced soils. Although T_1 is one of the treatments subjected to oxidation, it recorded the lowest ionic mobility throughout the trial period. In all the treatments, there was a slight increase in mobility between 1WAI to 4WAI; between 4WAI to 8WAI there was a considerable increase in mobility of all the elements (Ca, Mg, Na, and K). At 12WAI however, there was a decline in mobility for all elements across all treatments. The highest mobility was observed at 8WAI while the lowest at 1WAI. Generally, mobility of all elements was highest at T_{12} and lowest at T_1.

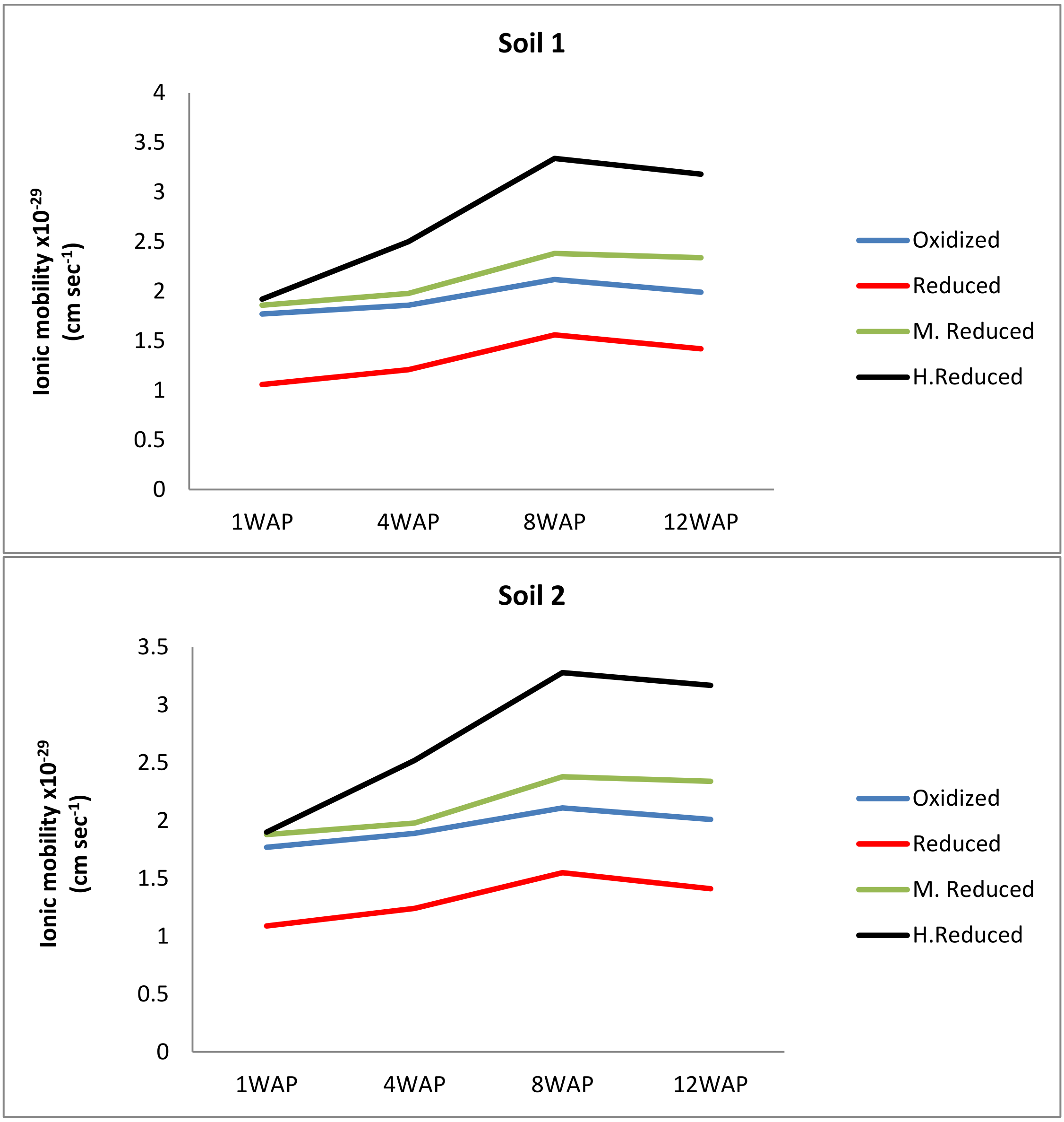


Figure 1. Effect of Redox Potential on Calcium Mobility over 12 Weeks

Figures 5 compares the mobility of exchangeable cations in the soil under oxidized and reduced conditions at 12 weeks after incubation. Calcium ion is represented by blue lines, magnesium ion by red, sodium ion by green and potassium ion by yellow. Throughout the trial period, potassium ion recorded the highest values for mobility across all treatments, followed by sodium, then calcium and magnesium recorded the lowest ionic mobility. Figures 6 to 9 compare the ionic mobility between the two soils used during the trials. Figure 6 shows the calcium ion mobility, figure 7 shows that of magnesium ion, figure 8 shows sodium ion mobility and figure 9 shows potassium ion mobility. Soil 1 was a sandy clay loam and is represented by blue lines on the graph while soil 2 was a clayey loam represented by red lines on the graph. Mobility was faster in soil 1 than soil 2 on all treatments at all levels.

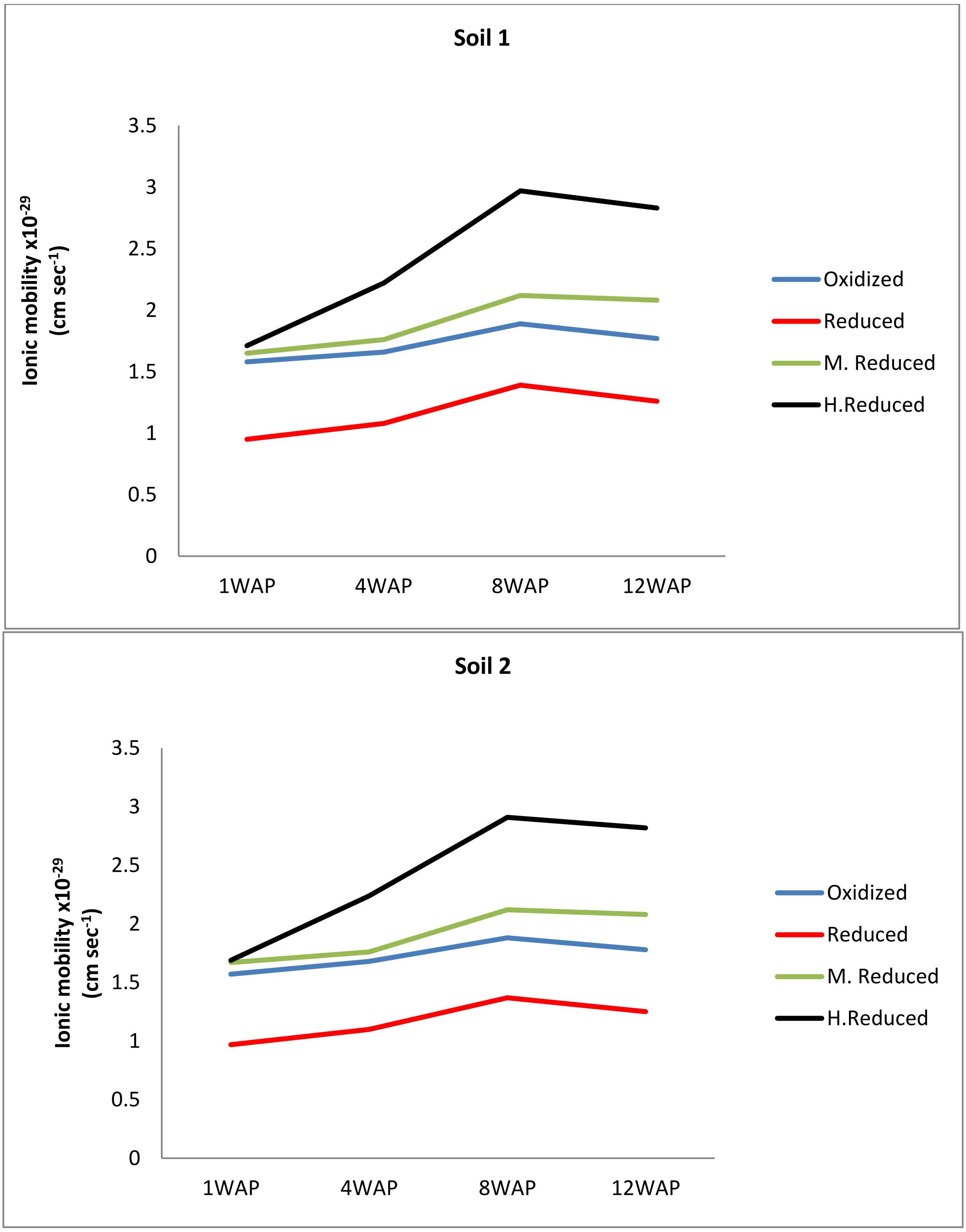


Figure 2. *Effect of Redox Potential on Magnesium Mobility over 12 Weeks*

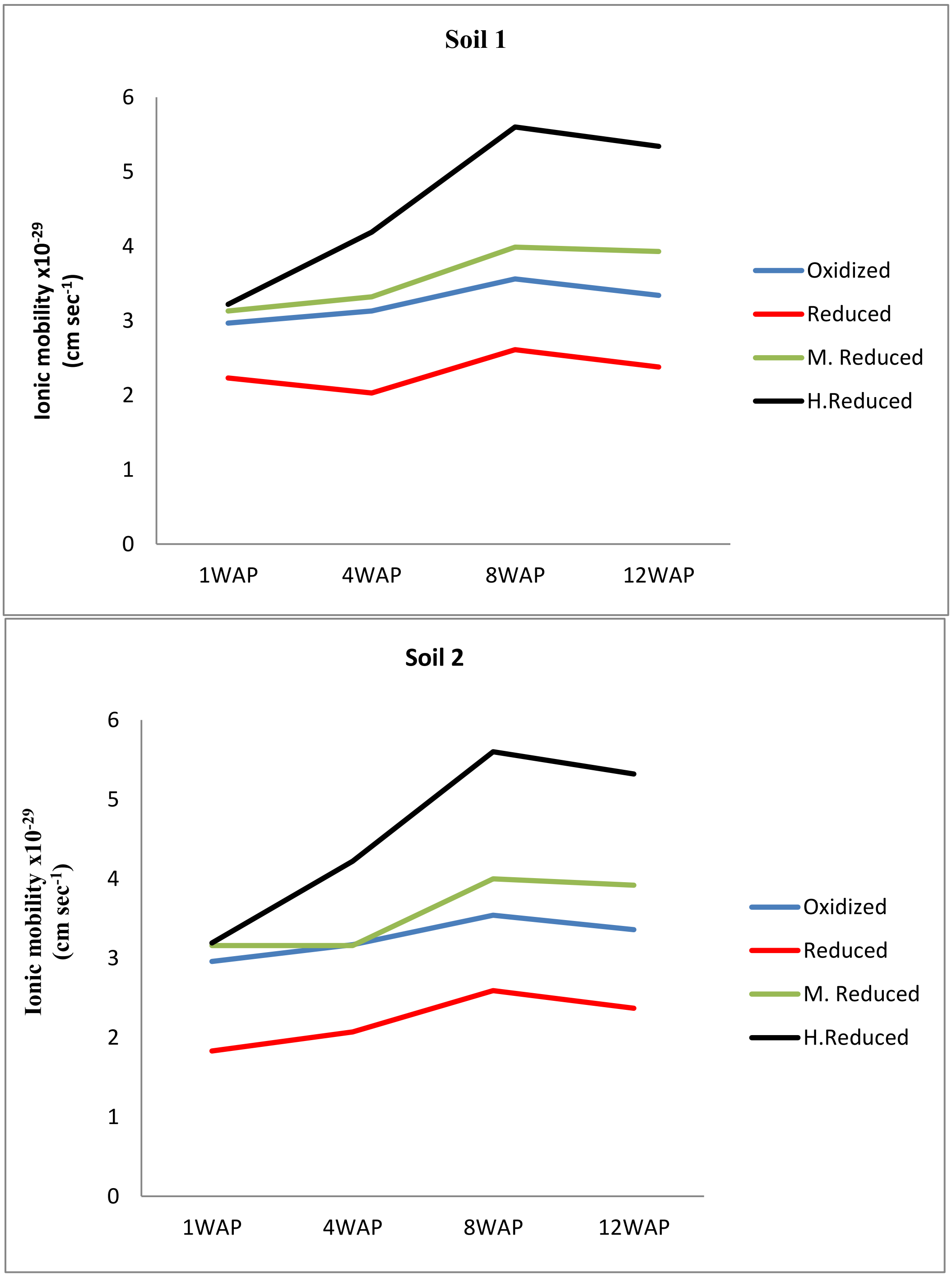


Figure 3. *Effect of Redox Potential on Sodium Mobility over 12 Weeks*

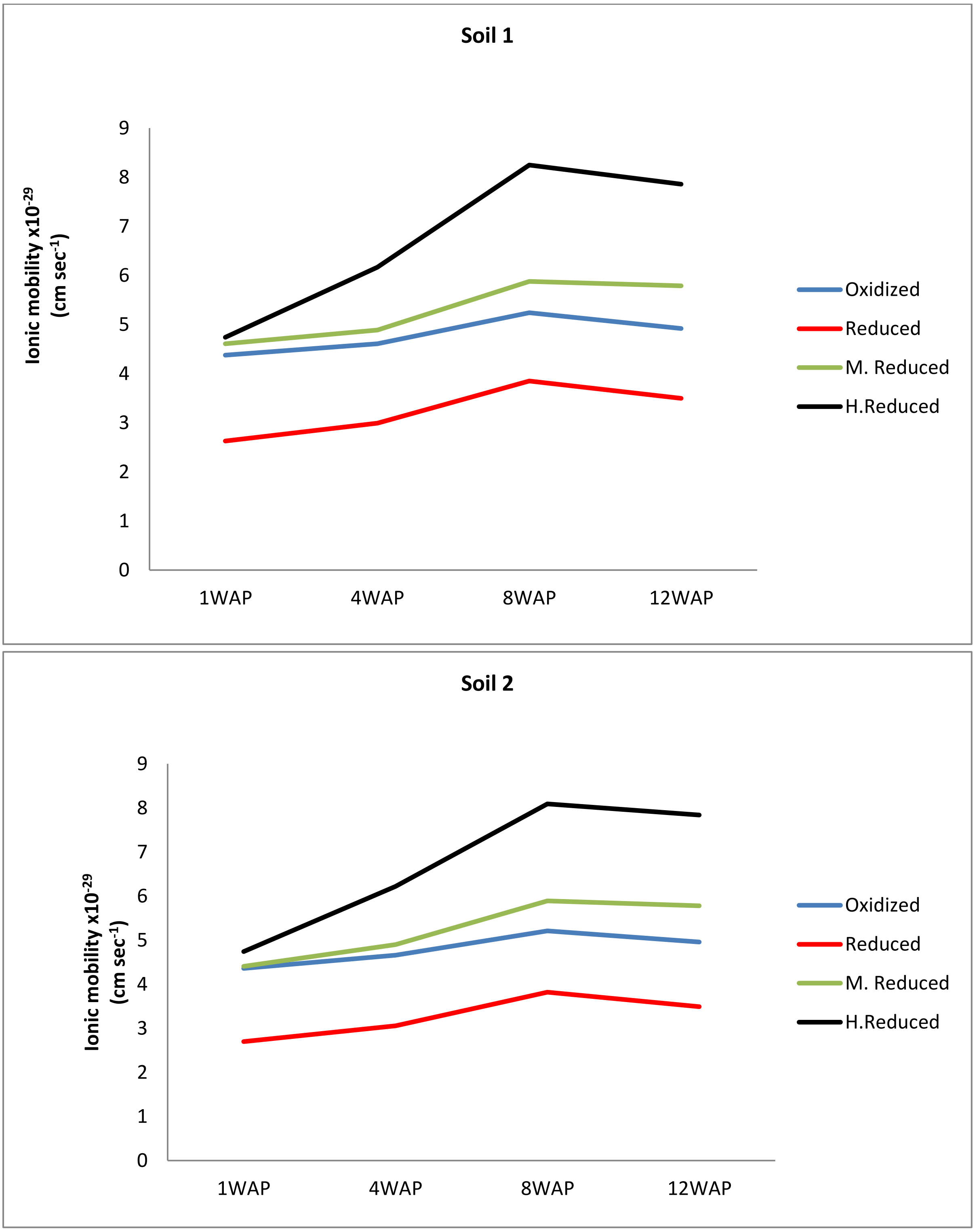


Figure 4. *Effect of Redox Potential on Potassium Mobility over 12 Weeks*

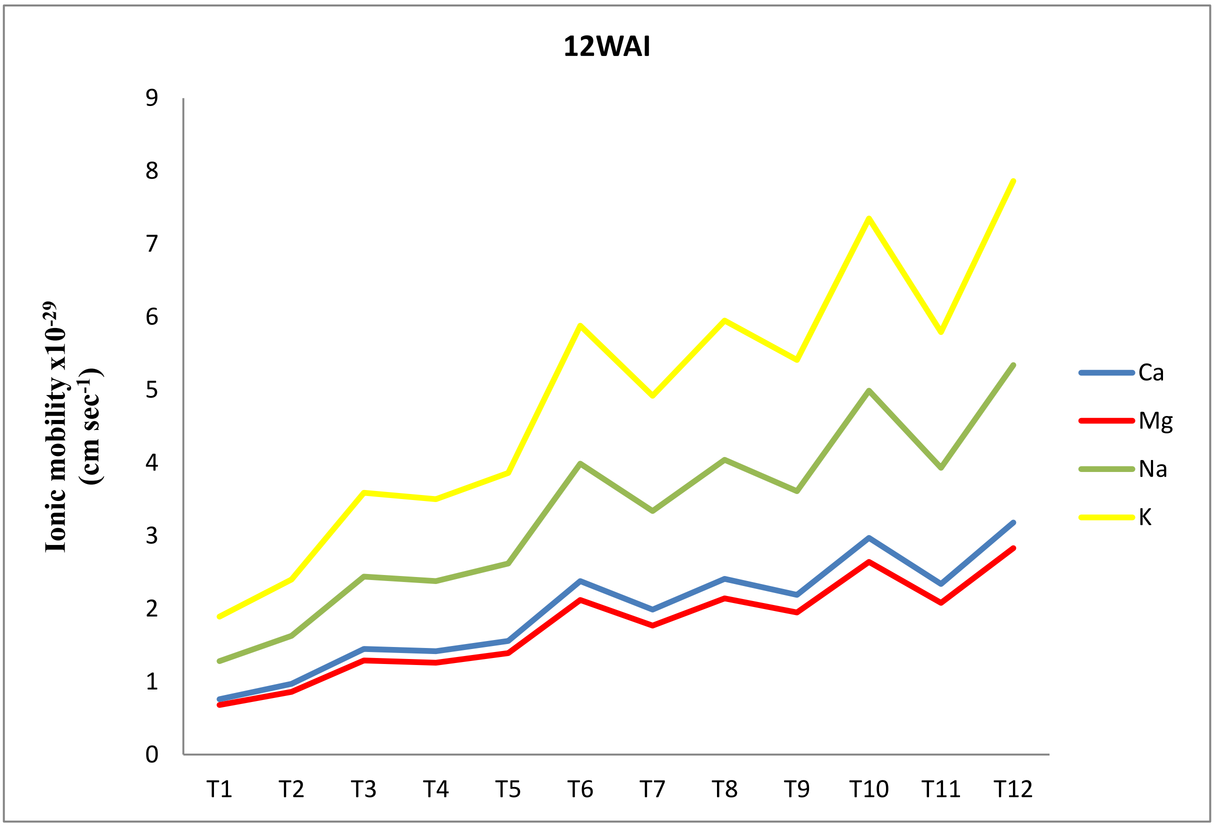


Figure 5. *Comparing Soil Cation Mobility at 12WAI*

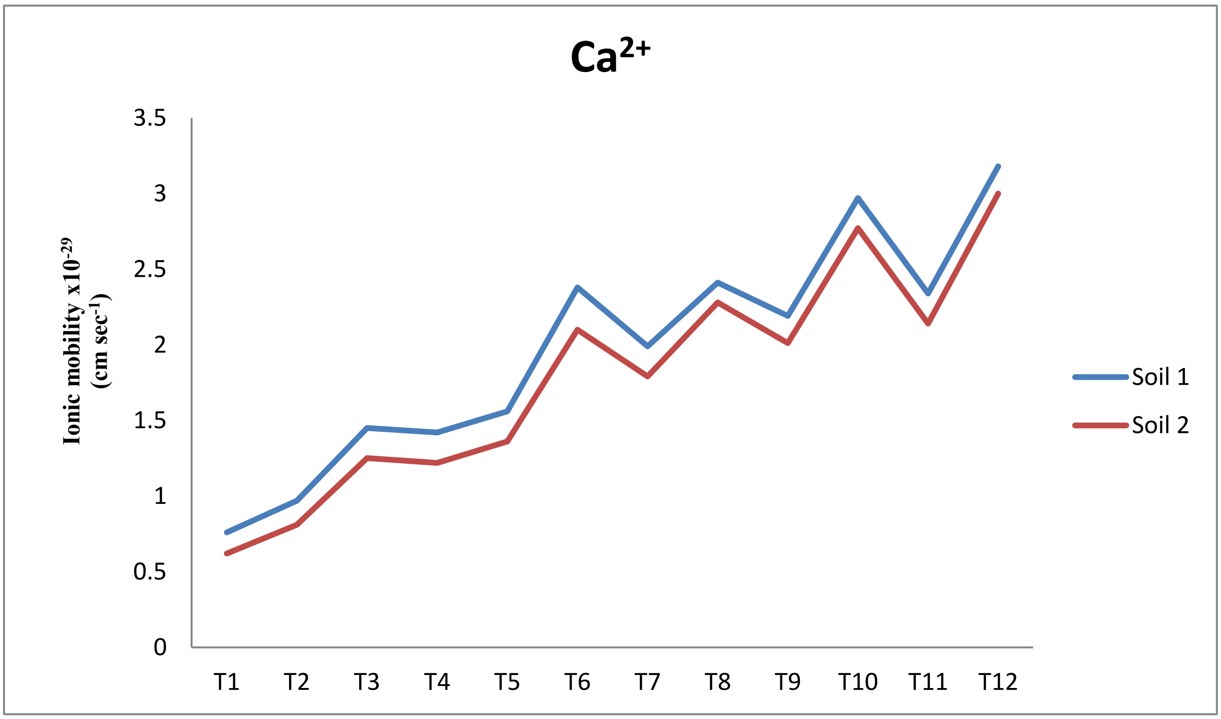


Figure 6. *Comparing Ionic Mobility between the two Soils (Ca^{2+})*

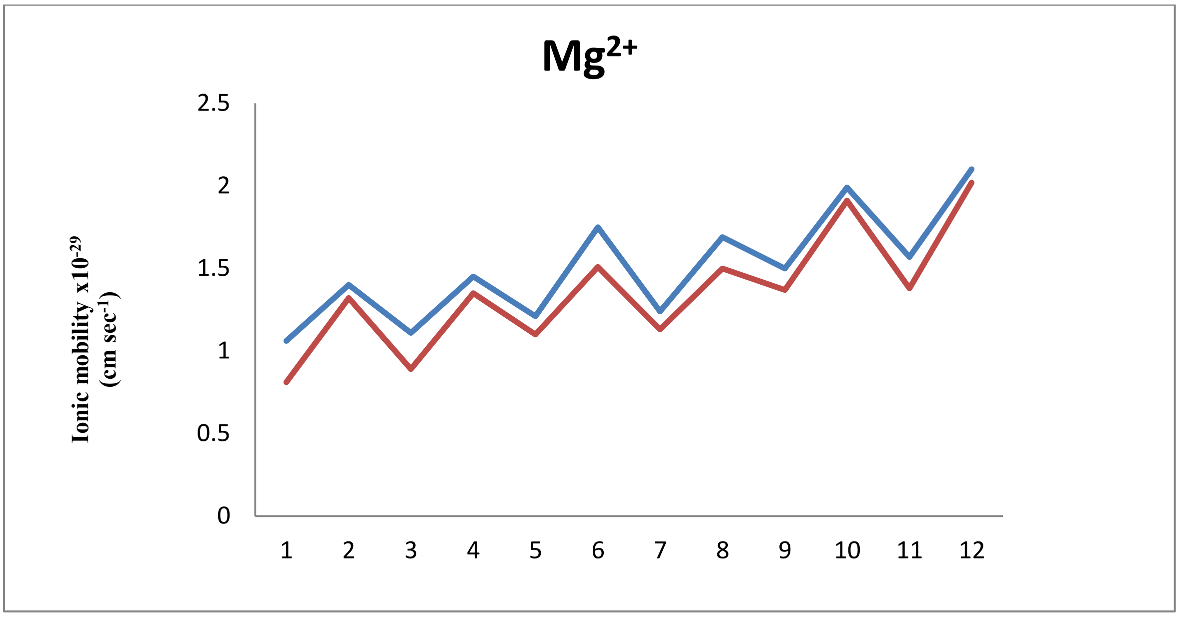


Figure 7. *Comparing of Ionic Mobility between the two Soils* (Mg^{2+})

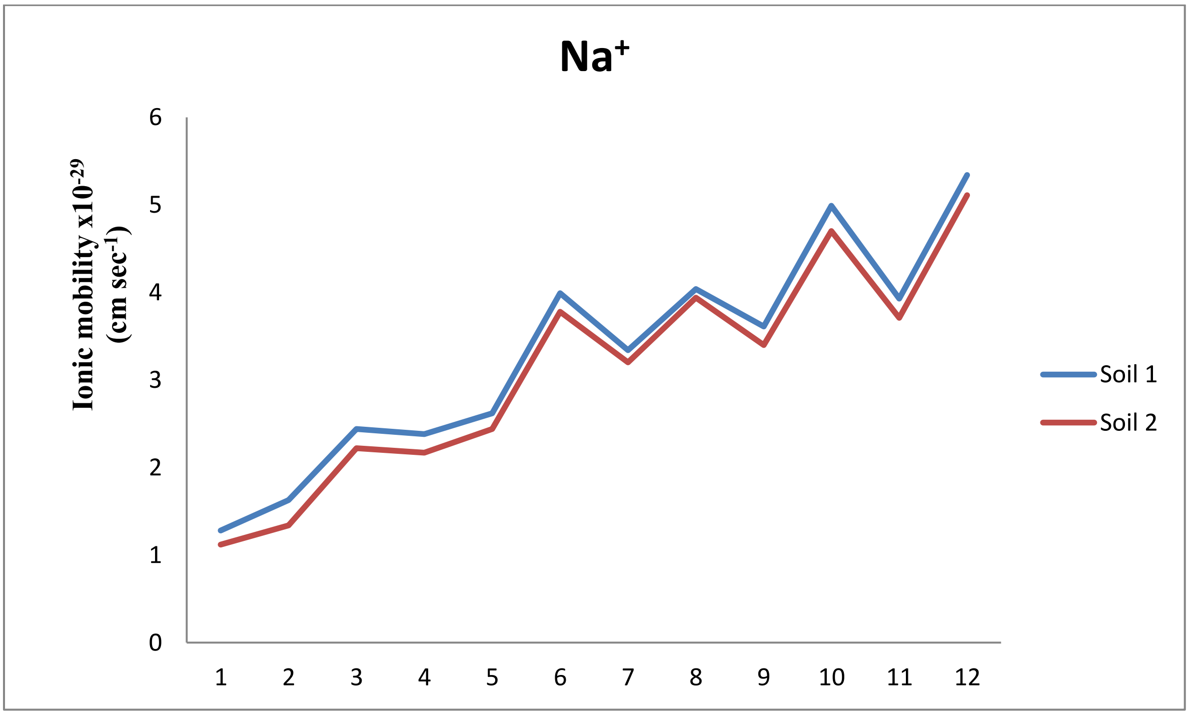


Figure 8. *Comparing of Ionic Mobility between the two Soils* (Na^{+})

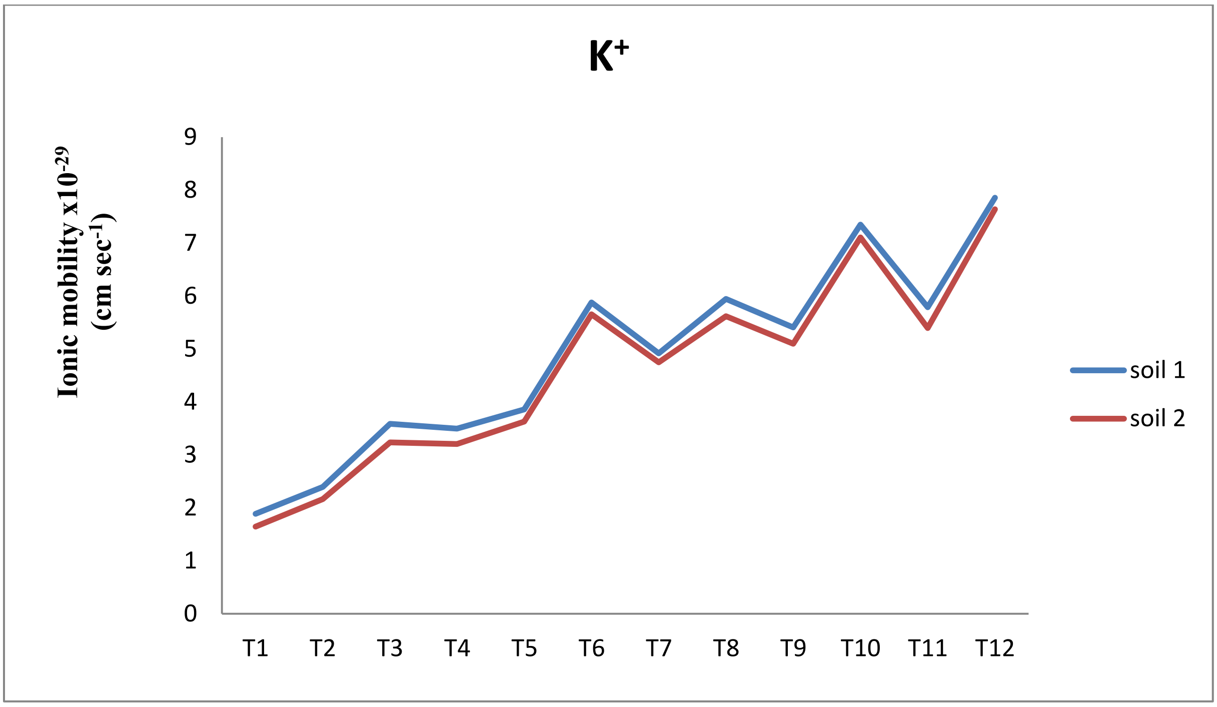


Figure 9. *Comparing of Ionic Mobility between the two Soils (K^+)*

Discussion

Ionic mobility is the average velocity with which an ion drifts through a specified gas under the influence of an electrical field. The results from this study showed that reduction processes favor mobility of ions in the soil. When an electric field is applied to ions in a medium (a phenomenon called electrophoresis), it causes the positive ions to move with the electric field (Dibyadeep, 2010). Under reduced conditions, electrons are transferred from organic matter to the soil components (Bi et al., 2013), these process of electron transfer generates electric charges in the soil solution which in turn creates an electric field (Bhadra, 2015). It then follows that the more reduced the environment becomes, the greater the electric field and consequently, the faster the ions move along the electric field created. This is because the electric field will exert a force that accelerates the charged particle (Daniel, 2014). This agrees with the work of Secco 2011 who stated that among other things, charge carrier concentration contributes to ionic movement. It was also found out that potassium ion K^+ had the highest movement in the soil solution followed by sodium ion Na^+ followed by calcium ion Ca^{2+} and the slowest is magnesium ion Mg^{2+}. This can be attributed to the fact that electrical currents are created during electron transfer (Bhadra, 2015), when the soil environment is more negatively charged, the ionic radii of the cations increase and the ions become bigger in size (Socratic, 2014). The size of ions is a crucial factor that determines the mobility of ion. In water, these ions are surrounded by water molecules because water is a dipole which is attracted towards positive charges of these ions (Bert, 2018).

With increase in size, hydration power or tendency for ion to be hydrated decreases, however, as the ion gets smaller their surface area increases and the tendency for it to be hydrated or attract more water molecules increase (Aftab, 2020). Due to more water molecules surrounding it, its mobility in water is impeded because it will have to carry water molecules with it as it moves. Potassium according to the periodic table, is the largest of the cations and becomes even bigger under reduced conditions because it has lesser amount of proton in its nucleus (Earles, 2015), this makes the force of attraction between its orbital electron and nucleus not as strong as obtained in other cations. This is followed by sodium then calcium and lastly magnesium which is the smallest. Mobility was also found to be higher in sandy clay loam than clay loam. Another major factor that affects ionic mobility is the amount of free spaces in the soil aggregate. The more compact a soil is, the lower the movement of ions and the less compact a soil is, the faster the ions move. This agrees with the work of Murawski, (2017) who stated that ionic conductivity is affected by size of particles, ionic mobility is a function of conductivity. Given that sandy clay loam has more pore spaces than clay loam, it makes it faster for ions to move in them than the clay loam. This also corroborates the findings of Nawaz et al., 2012 who concluded in their work that compaction of soil reduces mobility.

Conclusion

This study has confirmed that redox status of soil influences soil ionic mobility. Agricultural practices that encourage the use of organic matter as soil amendments can induce reduction processes and hence ionic mobility of cations in the soil. The study further revealed that potassium is the most mobile element in the soil and as such readily available to plants however care must be taken to avoid it leaching out of the vadose zone of the soil because of its high mobility. Sodium was also found to be highly mobile under reduced conditions; this knowledge can be used in the remediation of sodic soils to reduce the salt content. However, it is recommended that this experiment should be repeated for other soil elements under other textural classes as this would be relevant in soil management and conservation.

Declaration of Interest Statement

I declare that there is no conflict of interest of any kind between the authors of this manuscript.

References

Aftab, D. 2020. Hydration Energy Definition & Meanings : A Complete Guide. Guidance Corner. https://guidancecorner.com/hydration-energy/.

Akingbola, O.O., A.J. Adeyemo, S.O. Oladele, and S.O. Ojeniyi, 2016. Physical Status and Infiltration Dynamics of Tropical Alfisol of South- Western Nigeria as Affected by Poultry Manure. Applied Tropical Agriculture. 21(3):102-111. www.futa.edu.ng/journal/home/volumep/5/11.

Alexander, M. 1964. Biochemical ecology of soil microorganisms. Annual Review of Microbiology Volume 18: pp 217–250.

Amodu, T. O., G. O. Dayo-Olagbende, and O. O. Akingbola, 2019. Effect of Selected Organic Residues and Inorganic Fertilizers on the Performance of Okra (Abelmoschus esculentus). Sustainable Food Production. 5:17-23.

Bert, M. 2018. Are Ions Hydrophobic or Hydrophilic? Retrieved from sciencing.com/are-ions-hydrophonics-or-hydrophilic-13710245 on 9th January, 2020.

Bhadra N. 2015. Physiological Principles of Electrical Stimulation. In Implantable Neuroprostheses for Restoring Functions. Woodhead Publication Series in Biomaterials, pp 13-43.

Bi, R., Q. Li, W. Yu, Y. Yuan, Z. Shungui, 2013. Electron Transfer Capacity of Soil Dissolved Organic Matter and its Potential Impact on Soil Respiration. Journal of Soils and Sediments 13(9).

Brady, N.C., R. R. Weil 2010. The nature and properties of soils.

Brzezinska, M. 2004 Aeration Status of Soil and Enzyme Activity. In Glinski J., Josefaciuk G. Stahr K. (eds) Soil Plant Atmosphere Aeration and Environment Problems. Agrophysics. Hohenhein University/ Institution of Agrophysics Polish Academy of Science, Lubli-Stuttgart, Pp 55-59.
https://doi.org/10.18052/www.scipress.com/SFP.5.17

Chadwick O.A., J. Chorover 2001. The chemistry of pedogenic thresholds. Geoderma Volume 100: pp 321–353.

Charyulu, P.B.B.N., and V.R. Rao 1980 Influence of various soil factors on nitrogen fixation by Azospirillum spp. Soil Biology and Biochemistry Volume 12: pp 343–346.

Chuan, M.C., G.Y. Shu, J.C. Liu, 1996. Solubility of Heavy Metals in a Contaminated Soil: Effects of Redox Potential and pH. Water, Air and Soil Pollution. 90:543-556.

De Gara L, V. Locato. S. Dipierro, M.C. De Pinto, 2010. Redox homeostasis in plants. The challenge of living with endogenous oxygen production. Respiratory Physiology and Neurobiology Volume 173: pp S13–S19.

Dessaux, Y., P. Hinsinger and P. Lemanceau, 2009 Rhizosphere: so many achievements and even more challenges. Plant Soil volume 321: pp 1–3.

Dibyadeep ,P. 2010. Focussed electric field induced ion transport: A novel nano-patterning process. IOWA State University Capstones, Theses and Dissertations.

Dietz, K. J. 2003. Redox Control, Redox Signaling and Redox Homeostatic in Plant Cells. Int.Rev. Cytol Vol 228: 141-193

Drew, M.C., P.H. Nye and L.V. Vaidyanathan, 1969. The Supply of Nutrient Ions by Diffusion to Plant Root in Soil: Absorption of Potassium by Cylindrical Root of Onion and Leek. Plant and Soil 30(2):252-270. www.jstor.org/stable/42946744.

Earle, S., 2015. Electrons, Protons, Neutrons, and Atoms – Physical Geology. Pressbooks. https://opentextbc.ca/geology/chapter/2-1-electrons-protons-neutrons-and-atoms.

Ehsan T., R., K.M. Pichu. Gleen, 2010. High concentration of Na+ and Cl- ions in Soil Solution Have Simultaneous Detrimental Effects on Growth of Faba Bean Under Salinity Stress. Journal of Experimental Botany, 61 (15):4449-4459 https://doi.org/10.1093/jxb/erq251.

Falkowski P.G., T. Fenchel. E.F. Delong, 2008. The Microbial Engine That Drives Earth’s Biogeochemical Cycle. Science Vol 320: 1034-1039

Foyer C.H. and G., Noctor, 2005. Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiology of Plant Volume 119: pp 355–364.

Greenberg, R.C., 1998 Understanding the redox (rH2) measurement of the biological terrain: Part I. Biomed Therapy volume 16: pp 156–158.

Heron, G., T.H Christensen, and J.C. Tjell, 1994. Oxidation capacity of aquifer sediments. Environmental Science and Technology 28:153-158.

Kemmou, S., J.E. Dafir, M. Wartiti and M. Taoufik, 2006. Seasonal variations and potential mobility of sediment phosphorus in the Al Massira reservoir, Morocco. Water Quality Research Journal Canada Volume 41: pp 427–436.

Kimbrough D.E., Y. Kouame, P. Moheban S. Springthorpe, 2006 The effect of electrolysis and oxidation-reduction potential on microbial survival, growth and disinfection. Internaltional Journal of Environmental Pollution Volume 27: pp 211–221.

Kralova M., P.H. Masscheleyn, W.H. Patrick Jr., 1992. Redox Potential as an indicator of Electron Availability for Microbial Activity and Nitrogen Transformation in Aerobic Soils. Z Mikrobiology Vol 147: pp 328-399.

Lambers H., S.F.I. Chapin, and T.L. Pons,,2008. Plant physiological ecology. Springer, New York.

Lovley D.R., J.L. Fraga, E.L. Bohl, L.A. Hayes, E.J.P. Phillips and J.D. Coates, 1998. Humic Substances as a Mediator for Microbially Catalyzed Metal Reduction. Acta Hydrochimica et Hydrobiology Volume 26: pp 152–157.

Marion D., C. Pacciariello, A. Puppo, P. Frendo, J.P. Jacquot 2009. The Redox State, A Referee of the Legume Rhizobia Symbiotic Game. Advanced Botany Research Vol 52: 115-151.

Mathis, P. 1995. Photosynthesis: from Light to Biosphere In Proceedings of the 10th International Photosynthesis Congress Volume 3, Montpellier, 20–25 August 1995, France.

Murawski, D. 2017. Effect of Particle Size and Pretreatment on the conductivity of Glass Powder during Compaction. De Gruyter. https://www.degruyter.com/document/doi/10.1515/zpch-2016-0926/html

Nawaz, M. F., G. Bourrié, and F. Trolard, 2012. Soil compaction impact and modelling. A review. Agronomy for Sustainable Development. https://link.springer.com/article/10.1007/s13593-011-0071-8.

Oglesby, J. P., 1997. Redox buffering by soil organic matter. Abstract of Papers of the American Chemistry Society Volume 213: pp 147-156.

Phillips, I.R., 1998. Phosphorus Availability and Sorption under Alternating Waterlogged and Drying Conditions. Communications in Soil Science and Plant Nutrition Volume 29: pp 3045–3059

Rabenhorst, A.C., W.D. Hively and B.R. James, 2009. Measurements of soil redox potential. Soil Science Society of America Journal Volume 73: pp 668–674.

Rayment G.E. and D.J., Lyon, 2011. Soil Chemical Methods: Australasia.

Reddy, K.R and R.D., Delaune, 2008. Biogeochemistry of Wetland Science and Applications. CRC Press, Boca Raton

Roba, T.B. 2018. Review on: The Effect of Mixing Organic and Inorganic Fertilizer on Productivity and Soil Fertility. Open Access Library Journal 5(6) pp 1-11.

Sallade, Y.E. and J.T. Sims, 1997. Phosphorus transformations in the sediments of Delaware's agricultural drainage ways: II. Effect of reducing conditions on phosphorus release. Journal of Environmental Quality Volume 26: pp 1579–1588.

Secco, E. 2011. Factors Affecting Ion Transport in Fast Ion Conductivity Solid. MRS Online Proceeding Library Archive 293.

Simek M., J.E. Cooper, 2002. The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. Eur. J. Soil. Sci. 53, 345–354.

Singh, B. and D.G. Schulze, 2015. Soil Mineral and Plant Nutrition. Nature Education Knowledge 6(1):1

Socratic. 2014. How does an electric field affect the movement of a charge? Socratic.Org.https://socratic.org/questions/how-does-an-electric-field-affect-the-movement-of-a-charge

Timsina, J. 2018. Can Organic Sources of Nutrient Increase Crop Yield to Meet Global Food Demand? Agronomy 8: 214. https://doi.org/10.3390/agronomy8100214.

Vadas P.A. and J.T. Sims, 1998. Redox status, poultry litter, and phosphorus solubility in Atlantic Coastal plain soils. Soil Science Society of America Journal Volume 62: pp 1025–1034.

Influence of Forest Vegetation on Organic Matter Composition after Restoration of Mining Spoils in the Town of Pernik

Vania Kachova
Forest Research Institute – Bulgarian Academy of Sciences
Bulvd. "Kliment Ohridski" 132, Sofia, 1756, Bulgaria
Corresponding Author: Vania Kachova, e-mail: vania_kachova@abv.bg

Received: 9 September 2020 *Accepted: 1 March 2021*

Abstract

Three tree species are studied: coniferous plantation of black pine / *Pinus nigra* Arn./ and deciduous: birch / *Betulla alba* L./ and ash / *Fraxinus pennsylvanica* Marsh/, on the territory of Baikusheva neighborhood in Pernik, near the mine "M. Tolbuhin ", after performed 45 years ago reclamation. The accumulation of organic matter in the mining spoil and humus composition are analysed. High amounts of org.C are found under all three types of woody plants (tC% = 3.65 ÷ 9.50 in top layers 0-20cm). This is also due to the presence of large amounts of coal carbon particles in the substrate. Humus accumulation is more pronounced under deciduous species and especially under the newly studied species of *Fraxinus pennsylvanica* Marsh (Humus% = 11.42 ÷ 16.38). But here the process of humification takes place to a lesser extent (~ 10 ÷ 16%). On the contrary, under the coniferous plantation of *Pinus nigra* Arn. is accumulated less org. C (tC% = 1.34 ÷ 3.65), but the humification processes are going in the highest degree (~ 18 ÷ 32%). However, this is also associated with the formation of the largest amounts of the more mobile parts of the organic matter: fulvic acids (Cf ~ 11% from tC). Among deciduous species under *Fraxinus pennsylvanica* Marsh, fulvic acids move in the range of the lowest values (4-5% of tC).

Key words: recultivation, organic carbon, humic and fulvic acids

Introduction

Mining activity is associated with the destruction of natural soil cover, transportation and deposition of soils in the form of huge heterogeneous masses of soil substrates - mining spoils. Restoring their ecological characteristics and recovering a good growing environment is an important and "hot" issue facing scientists for decades (Prokopiev, 1967; Donov et al., 1978; Banov et al., 1989; Zheleva et al., 2004; Atanassova et al., 2018; Simeonova et al., 2018; Nikova et al., 2019). It is generally believed that the best cost-effective and most applicable method is a phytoremediation (Henry et al., 2013; Deng et al., 2020). In this method with particular importance is the land-use type, kind of vegetation: forest, grass or agricultural plants (Banov, Marinova, 2016.). Forest vegetation is especially valuable in case of heavier pollution of the substrate and for building a high aesthetic value of the landscape with remediation applicability. In addition, with its deep roots and huge forest litter, the forest vegetation significantly and in depth improve the characteristics of the substrate (Gencheva and Filcheva, 1995). Here the question of the specificity of the species of forest-tree vegetation is important (Feng et al., 2019). Aboveground vegetation closely correlated with soil properties including soil organic carbon (Sun et al., 2019; Jobbagy, Jackson, 2000). Who are the best phytoremediators among forest-tree species remains an open question, for which there are not many studies (Gencheva and Filcheva, 1995). The influence of the species on the content and composition of the soil organic matter (SOM) is especially indicative. It is that driver of soil processes which is of main importance for biochemical characteristics of soil and especially of its quality. Afforestation affects the content of SOM and its composition (Zhiyanski et al, 2012) especially on substrates of mining activity (Bech et al., 2012; Sokolovska et al., 2014). Analyses of variance shows that all soil properties including pH, total C and total N influence by tree species (Zhiyanski et al, 2008).

In our country in the Pernik mining region, as one of the biggest in the country, these issues also arise. A number of scientists have worked here on the composition of mining substrates (Ivanov, 2007; Sokolovska et al., 2014; Hristov et al., 2015), contents and composition of SOM (Ivanov et al., 2007; Gencheva and Filcheva, 1995; Filcheva, 2000) and interaction of tree vegetation with soil`s substrates (Kolarov and Shishkova 1983; Gencheva and Filcheva, 1995; Ilinkin and Dimitrova, 2019). It is important to see the influence of forest-tree species on humus accumulation and the composition of humus over a longer period of time, as the age of the substrate is also a factor influencing soil formation processes (Ivanov, 2007).

The aim of the present study is to analyze the influence of *Pinus nigra Arn.*, *Betula alba* L.and *Fraxinus pennsylvanica* Marsh on the accumulation and composition of organic matter in spoils from mining sites of Pernik.

The study has both confirmative character for the species *Pinus nigra* Arn, *Betula alba* L., but having in mind that it is made in a significantly older and unexplored substrate for this purpose, as well as the accumulation of new information regarding the species *Fraxinus pennsylvanica* Marsh.

Material and methods

The sample plots (SPs) are set on a reclaimed spoil in the area of Baikusheva mahala, about 200 m from the mine “M. Tolbuhin”, at the exit from Pernik to the right of the road Pernik - Breznik. The recultivation was carried out in the early 70's and the plantations are about 45 years old. There are 3 SPs under the different types of woody plants:

SP1 (sample plot 1) – under black pine /*Pinus nigra* Arn./ in the middle of the hill, in the presence of grass cover of cereals ~ 60%;

SP2 (sample plot 2) – under birch /*Betula alba* L./ at the bottom of the hill, in the presence of grass cover of cereals ~ 90%;

SP3 (sample plot 3) – under ash /*Fraxinus pennsylvanica* Marsh/ at the top of the hill, in the presence of grass cover of cereals ~ 30%;

Parents materials are represented mainly by marls, with impurities of black coal shale, as well as slag (Hristov et al., 2015). Sampling was performed in 3 layers: 0-20; 20-40; 40-60 cm. It is determined: pH – potentiometrically by pH-meter "Placitronic, MV 88" according ISO 10390, 2002; Total carbon (C%) and humus (%): by the Thurin method; Total nitrogen (N%): by the Keldal method – (Donov et al., 1974); the composition of humus by Kononova, Belchikova method (1961). This method consists of determination of total content of humic and fulvic acids with a mixed solution of 0.1N $Na_4P_2O_7$ and 0.1M NaOH; free acids and bound to the sesquioxides (R_2O_3) with 0.1M NaOH; aggressive fulvic acids with 0.05M H_2SO_4.

Results and Discussion

The results of the study of the main chemical properties of the spoil`s substrate under the different forest-tree species are given in Table 1..

With recultivation after mining activity is associated the changes in pH of substrate (Cardenas-Aguiar et al., 2020). All substrates of investigated SPs has pH in the range 7.05 ÷ 7.60 which defined substrates as neutral. This result coincides with the characteristics of most spoilss of the Pernik mining basin, found by Donov et al., (1978). In literature dominates opinion that more often vegetation restoration of mining sites could alleviate soil alkaline and in such way moderate accumulation of soil nutrients (Feng et al., 2019; Deng et al., 2020). In our cases acidity is stabilized around pH = 7. For tree species pH preferences vary between range 5.0-6.5 (Feng et al., 2019), which shows a slightly more alkaline reaction of investigated substrate from favorite of the species.

Table1. *Main characteristics of mining spoil in SPs*

SPs	Depth cm	pH	Humus%	C%	N%	C/N
under black pine /*Pinus nigra* Arn./						
SP1	0-20	7.05	6.29	3.65	0.17	21.47
	20-40	7.47	4.96	2.88	0.14	20.57
	40-60	7.60	2.32	1.34	0.08	16.75
under birch /*Betula alba*/						
SP2	0-20	7.23	11.32	6.91	0.28	24.68
	20-40	7.30	11.09	6.43	0.23	27.96
	40-60	7.34	10.09	5.86	0.20	29.30
under plain /*Fraxinus pennsylvanica*/						
SP3	0-20	7.26	16.38	9.50	0.35	27.14
	20-40	7.27	15.23	8.83	0.29	30.45
	40-60	7.31	11.42	6.62	0.25	26.48

The main indicator determining soil fertility for rehabilitated mining substrates is organic matter (Hristov et al., 2015). It plays an important role in soil formation processes in mining areas (Sokolovska et al., 2014). Tree species influence SOM, tN and C/N in top-soils 0-10cm (Zhiyanski et al., 2008). In our case, there is a significant and differentiated differences of the influence of tree species on the accumulation of humic substances in the substrate. The substrate in terms of humus content is defined as rich humu (Filcheva and Ilieva, 2014), and the one under ash /*Fraxinus pennsylvanica* Marsh/ as very rich in humus (humus > 15%). Main component in OM (organic matter) is OC (organic carbon) that plays crucial role in soil structure and fertility (Feng et al., 2019). The content of OC varies moderately to high under black pine /*Pinus nigra* Arn./ to very high under birch /*Betula alba* L./, and especially under ash /*Fraxinus pennsylvanica Marsh/* (OC > 5.9%). This high content of OM and OC in spoil is due to the availability of coal dust in the substrata, which is an inherited coal carbonized organic matter, and which is not related to the newly accumulated due to the decomposition of organic waste from tree species. The same effect of coal particles on tC is found by other authors studying the organic matter in mining spoils of Pernik (Donov et al., 1978; Gencheva and Filcheva, 1995; Ivanov et al., 2007; Sokolovska et al., 2014; Hristov et al., 2015). In our case however, having in mind that this is one and same mining spoil, which suggests a similar composition, including organic composition, and having in mind that the content of OM under coniferous species (black pine) is differentiated lower, we could talk about the influence of tree species on organic accumulation. Very high content of organic matter is found under deciduous tree vegetation in mining substrates from the vicinity of the studied spoil Hristov et al., (2015). Donov et al. (1978) for reclaimed spoils from Pernik found the highest accumulation of organic matter under birch /*Betula alba* L/.. The same was reported by Kolarov, Shishkova (1983). In our case, there is indeed a very high content of OM under birch, but especially high is the accumulation of organic matter under the newly studied species – ash /*Fraxinus pennsylvanica* Marsh/.

The content of tN follows the trend of tC. In the surface layers, the substrate under black pine is moderately stored with tN, but poorly stored at a depth of 20-40 cm (tN <0.15%). On the contrary, under birch it is well stocked with tN (0.2-0.25%). Very stocked with tN (> 0.25%) is the substrate below clear /*Fraxinus pennsylvanica* Marsh/. This increased values of tC and tN and accumulation of litter and root biomass accelerate formation of humus (Mukhopadhyaya et al., 2013).

The other tendency that can be observed is the reduction of content of tC and tN into depth. This speaks about advanced degree of soil-forming process, because there are other older data about heterogeneity in distribution of tC into depth in more younger substrates from Pernik (Gencheva and Filcheva (1995). In Pernik, under tree species is also observed elementary soil forming process under 5-10 years after recultivation of mining spoils (Prokopiev, 1967; Gencheva and Filcheva, 1995). In literature has data that soil-forming process can be observed after 10 years and could be stabilized after 20 years (Feng et al., 2019). After 45 years here we find such stabilization of soil forming processes.

The results of the analysis of the composition of the organic matter in investigated spoil in Pernik is given in Table 2. In fact the composition of humus represents the ratio between humic acids - more stable part of humus which shows his maturity, and fulvic acids - more reactive and mobile part of humus, which shows his lability. In all depths in all SPs we find bigger quantity of humic acids in comparison with fulvic acids. This could be sign of more advanced degree of humus-accumulation processes as well as favorable climatic conditions. A higher percentage of humic acids under black pine is seen compared to deciduous tree species, but this is due to the higher percentage of total carbon extracted with the pyrophosphate solution. Ths is a peculiarity, that the lowest percentage of fulvic acids (~ 5%) is observed under the newly studied deciduous species /*Fraxinus pennsylvanica* Marsh/. The other trend is the decreasing of humic acids towards the deeper layers. In relation with fulvic acids this tendency has exception – in SP2. We calculated also the ratio Ch / Cf, which defines the type of humus. The humus is of the fulvate type at Ch / Cf < 0.5, of the humate-fulvate type at Ch / Cf > 0.5, and of the humate type at Ch / Cf > 1 (Orlov, 1985).

Table 2. *Composition of organic matter*

SP	Depth cm	Organic C% Extracted with 0.1 M $Na_2P_4O_7$ + 0.1MNaOH			Ch / Cf	Organic C% Fractions of humic acids		Non extracted organic C % (humin)	"Aggressive" fulvic acids
		Extracted totally	Humic acids Ch	Fulvic acids Cf		Free or bounded with R_2O_3	Bounded with Ca		
under black pine /*Pinus nigra* Arn./									
SP1	0-20	$\underline{1.34}$ 36.71	$\underline{0.76}$ 20.82	$\underline{0.44}$ 12.50	1.72	100	0	$\underline{2.32}$ 63.56	$\underline{0.12}$ 3.28
	20-40	$\underline{0.82}$ 28.47	$\underline{0.53}$ 18.40	$\underline{0.21}$ 7.29	2.52	100	0	$\underline{2.01}$ 69.79	$\underline{0.06}$ 2.08
	40-60	$\underline{0.66}$ 49.25	$\underline{0.44}$ 32.84	$\underline{0.15}$ 11.19	2.93	100	0	$\underline{0.78}$ 58.21	$\underline{0.07}$ 5.22
under birch /*Betula alba* L./									
SP2	0-20	$\underline{1.74}$ 25.18	$\underline{1.08}$ 15.62	$\underline{0.51}$ 7.38	2.12	100	0	$\underline{5.08}$ 73.51	$\underline{0.13}$ 1.88
	20-40	$\underline{1.48}$ 23.07	$\underline{0.95}$ 14.77	$\underline{0.40}$ 6.22	2.38	100	0	$\underline{4.97}$ 77.29	$\underline{0.14}$ 2.17
	40-60	$\underline{1.28}$ 21.84	$\underline{0.73}$ 12.46	$\underline{0.45}$ 7.68	1.62	100	0	$\underline{4.55}$ 77.64	$\underline{0.09}$ 1.53
under plain /*Fraxinus pennsylvanica* Marsh/									
SP3	0-20	$\underline{2.14}$ 22.53	$\underline{1.53}$ 16.11	$\underline{0.52}$ 5.47	2.94	100	0	$\underline{7.38}$ 77.68	$\underline{0.13}$ 1.36
	20-40	$\underline{1.48}$ 16.76	$\underline{0.97}$ 10.99	$\underline{0.43}$ 4.87	2.26	100	0	$\underline{7.35}$ 83.24	$\underline{0.09}$ 1.02
	40-60	$\underline{1.40}$ 21.15	$\underline{0.96}$ 14.50	$\underline{0.34}$ 5.14	2.91	100	0	$\underline{5.22}$ 78.83	$\underline{0.12}$ 1.81

P.S. column 1, 2, 3, 4, 5, 6, 7 are given as a % to the weight of soil sample,
under line column 1, 2, 3, 4, 5, 6, 7 are given as a % to the total carbon (from table 1)

Gencheva, Filcheva (1995) studying the humus formation of the spoil "Teva" found that it proceeds differently under different types of phytocenoses, and under black pine they founf that the process is of the fulvate type. In our case, where we study a significantly older substrate, with a more advanced soil formation process, we find under black pine a humate type of humus formation, and the same is under deciduous tree plantations (Ch / Cf > 1) (Orlov, 1985).

Logically, the highest amount of "aggressive" fulvic acids is observed under the coniferous plantation of black pine (2 ÷ 5% from tC) compared to deciduous tree species. The lowest content of these most mobile organic acids is below ash /Fraxinus pennsylvanica Marsh/ where the percentage of Cf "aggressive" is moving ~ 1%.

For all layers humic acids is free or bounded with R2O3 sesquioxides and do not form stable complexes with Ca ions. This could due to the chemistry of parent materials and shows one more expressed mobility of OM towards deeper layers. On the contrary, for the mining spoils of the "Teva" mine, Gencheva and Filcheva (1995) found 100% bound to Ca ions.

The amount of non-extracted with pyrophosphate C% - so called "humin" is very high for all samples (63-83%). This is normal when studying mining spoils from Pernik, where the amount of coal fractions is too high (Ivanov, 2007). For soils under forest crops in urbanized parks Zhiyanski et al. (2012) calculate the degree of humification (Ch / tC) * 100% which is very weak (<10%), weak (10-20%) and medium (20- 30%). The degree of humification in the individual layers of the substrate from all SPs are given in Figs. 1.

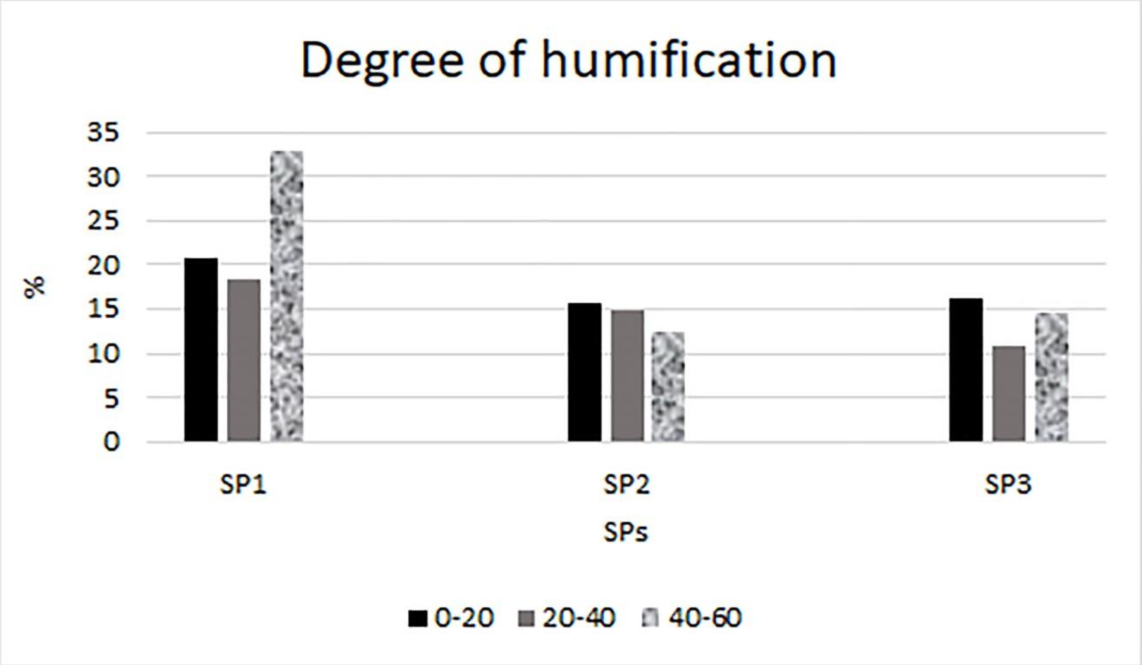


Figure 1. *Degree of humification in the spoils of investigated SPs*

The processes of humification take place with the highest degree under the coniferous plantation of black pine /*Pinus nigra* Arn./ Here, however, this is accompanied by a greater accumulation of more mobile fulvic acids. Under deciduous tree plantations, the degree of humification is lower, but nevertheless sufficient amounts of humic acids is accumulated, and less fulvic acids in comparison with coniferous plantation.

Conclusion

The current researches show that the processes of humus formation and humus accumulation in a mining spoil after a longer period of time from the conducted restoration (~ 45ys) take place differently under the different tree species. The largest amount of org.C accumulates under *Fraxinus pennsylvanica* Marsh. Although, under this plantation is the lowest degree of humification, in the substrate below it accumulate sufficient amounts of more stable humic acids, which have significantly high quantity in comparison with more mobile fulvic acids. Under the coniferous plantation of *Pinus nigra* Arn. although the rate of humification is the highest, it is associated with the formation of the largest amounts of fulvic acids, and therefore with a more labile composition of the newly formed humus in the mining substrate.

References

Atanasova, I., M. Banov, T. Shishkov, Z. Petkova, B. Hristov, P. Ivanov, E. Markov, I. Kirilov, M. Harizanova, 2018. Relationships between soil water repellency, physical and chemical properties in hydrophobic technogenic soils from the region of maritsa-iztok coal mine in Bulgaria. Bulgarian Journal of Agricultural Science, 24, 2: 10-17.

Banov, M., E. Filcheva, B. Hristov, 1989. Humus accumulation and composition of humus in reclaimed lands. Soil science and Agrochemistry, 4: 3-8. (in Bulgarian).

Banov, M., S. Marinova, 2016. Reclamation of Heaps of Solid Waste. – Ecological Engineering and Environmental Protection, 3: 23-31.

Bech, J., M. Sokolovska, M. Zhiyanski, E. Filcheva, N. Roca, 2012. Soil organic matter quality in a former mine site of NE Spain. Silva Balcanika, 13, 1: 30-37.

Cardenas-Aguiar, E., G. Suarez, J. Paz-Ferreiro, M.P.J. Askeland, A. Mendez, G. Gasco, 2020. Remediation of mining soils by combining Brassica napus growth and amendment with chars from manure waste. Chemosphere, 261 (2020) 127788, in press: www.elsevier.com/locate/chemosphere

Deng, J., X. Bai, Y. Zhou, W. Zhu, Y. Yin, 2020. Variations of soil microbial communities accompanied by different vegetation restoration in an open-cut iron mining area. Science of the Total Environment, 704, (2020), 135243 in press: www.elsevier.com/locate/scitotenv

Donov, V., Gencheva, S. & Yorova, K. 1974. Guidance on Soil Analysis, Sofia, Bulgaria: Zemizdat. p. 220. (in Bulgarian)

Donov, V., S. Gencheva, E. Geleva, N. Delkov, D. Pavlov, R. Milanov, 1978. Recultivation of Industrial Spoils. Zemizdat, Sofia, 166 p. (in Bulgarian).

Feng, Y. , J. Wanga, Z. Baia, L. Reading, 2019. Effects of surface coal mining and land reclamation on soil properties: A review. Earth-Science Reviews, 191: 12-25.

Filcheva, E., M. Noustorova, S. Gencheva, M. J. Haigh, 2000. Organic accumulation and microbial action in surface coal-mine spoils, Pernic, Bulgaria. Journal of Ecological Engeneering, 15, (1-2): 1-15.

Filcheva E., R. Ilieva. 2014. Soil organic matter of Bulgarian Chernozems. 17 th International Conference of IHSS, 1-5 September, Ioannina, Greece.

Gencheva, S., E. Filcheva, 1995. Organic matter composition in reclaimed lands from opencast mining. In: Proceedings from Sientific sessein “70 ys. Forestry education in Bulgaria”, 7-9 April, Sofia, 3: 3-10. (in Bulgarian).

Henry, H., J. Burken, R. Maier, L. Newman, S. Jerald, L. Schnoor, W. Suk, 2013. Phytotechnologies – Preventing Exposures, Improving Public Health. International Journal of Phytoremediation, 15, 9: 889-899.

Hristov, B., T. Shishkov, V. Katchova, E. Atanassova, I. Atanassova, 2015. Basic chemical and physic-chemical characteristics of soils and substrates in the region of Pernik coal mine basin. International Conference “Soils and agrotechnology in the changing worlds” 11-15 May, Sofia.

Ilinkin, V., V. Dimitrova, 2019. Forestry Reforestation vs. Spontaneous Revegetation-Soil Changes in Coal Mining Spoil Heaps Across Bulgaria. Ecologia Balkanica, 11, 2: 25-36.

Ivanov, I., 2007. Soil forming processes in reclaimed lands from recovered landscapes from industryal activities with different waies of usage. PhD Thesis, Institute of Soil Sci, Agrotechnology and Plant Protection “N. Pushkarov, 156 p.(in Bulgarian).

Ivanov, I., E. Filcheva, M. Banov, 2007. Organic matter content in Reclamed lands after Mining Industry. Soil science, Agrotechnology and Ecology, XLI, 1: 27–30. (in Bulgarian).

Jobbagy, E., Jackson, R., 2000. The vertical distribution of soil organic carbon and its relation to climate and vegetation. Belowgr. Process. Glob. Chang. 10: 423-436. https://doi.org/10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2.

Kolarov, D., R. Shishkova, 1983. Studies on phytosintetical activity of deciduous tree species growth after recultivation of “Maxim taban” in Pernik. Gorskostopanska nauka, XX, 6: 31-39. (in Bulgarian).

Kononova, M., N. Belchikova, 1961. Rapid method of mineral soil humus composition. Pochvovedenie, 10: 75-85. (in Russian).

Mukhopadhyaya, S., .S. Maitia, R Masto, 2013. Use of Reclaimed Mine Soil Index (RMSI) for screening of tree species for reclamation of coal mine degraded land. Ecological Engineerin, 57: 133-142.

Nikova, I., B. Hristov, N. Dinev, M. Hristova, 2019. Assessment of Soil Quality in a Copper Mining Region in Bulgaria. Ecologia Balkanica, 11(2): 13-23.

Orlov, D. C., 1985. Chemistry of soils. MGU, Moskva, 376 p. (in Russian).

Prokopiev, E., 1967. Landscaping of industrial regions. Zemizdat, Sofia, 144 p. (in Bulgarian).

Simeonova, T., M. Benkova, L. Nenova, I. Atanassova, 2018. Chemical Composition of Soil Solutions of Technosols from a Coal Mine Region in SouthEastern Europe. Bulgarian Journal of Soil Science, 3, 1: 4-12.

Sokolovska, M., M. Zhiyanski, E. Slavtcheva, N. Roca, J. Bech, 2014. General Characteristics of Organic Matter in Reclaimed Soils. 한국토양비료학회 학술발표회 초록집, 6: 509-509.

Sun, X., B. Li, F. Han, E. Xiao, Q. Wang, T. Xiao, W. Sun, 2019. Vegetation type impacts microbial interaction with antimony contaminants in a mining-contaminated soil environment. Environmental Pollution, 252: 1872-1881. (in Russian).

Zheleva, E., B. Bogdanov, M. Tsolova, 2004. New ecological and technical problems of reclamation of disturbed terrains from Maritza-Iztok coal mines. Management and Sustainable development, 1-2 (10), 323-328.

Zhiyanski, M., Kolev K., Sokolovska M., 2008. Tree species effect on soils from central Stara Planina Mountains. Forest Science, 4: 65-82.

Zhiyanski, M., M. Sokolovska, E. Filcheva, Y. Yordanov, 2012. Soil Organic Matter of Urban Forest Parks Journal of Agricultural Science and Forest Science, XI, 3: 21-26. (in Bulgarian).

Influence of Agro-Technical Treatments on Some Physical and Agrochemical Parameters of Haplic Vertisols

Vanya Lozanova, Ivan Dimitrov
"N. Poushkarov" Institute of Soil Science, Agrotechnologies
and Plant Protection,
7 Shosse Bankya Str., Sofia 1331, Bulgaria

Corresponding Author: Ivan Dimitrov, e-mail: idspasov@abv.bg

Received: 20 December 2020 *Accepted: 14 March 2021*

Abstract

By assessing basic agro-technical factors, optimal decisions can be made for good agricultural practice on soils with heavy soil texture, such as the Haplic Vertisols. The aim of the study is to identify the changes in the physical and agrochemical status of Haplic Vertisols that occur under the influence of applied agro-technical measures.

For the achievement of the purpose in the period 2016 -2019, field experiments, based on the block method in the Sofia Region on a Haplic Vertisols were carried out.

The study found out that soil moisture was a major limiting factor for crop development. The soil moisture content is influenced by the type of soil tillage, although the density of the weeds is also less affected by the parameter. The bulk density of the Haplic Vertisols is inversely correlated with the soil moisture content. In the case of deep loosening of maize, its values are reduced compared to those with plowing. Lower values were found for the parameter after plowing as a pre-sowing treatment for wheat, compared to the variants with disking.

The applied fertilization mainly affects the content of nitrate and ammonium nitrogen, the phosphorus forms remain with low values, it is clearly necessary to raise the norm and with a methodical approach it is necessary to achieve a sustainable level of absorbed phosphates. The content of the absorbed potassium is still satisfactory, and based on the data from the analysis, its reduction and export with the produced products are smaller.

Key words: Haplic Vertisols, agricultural treatment, physical and agrochemical properties

Introduction

The problems of agriculture in the separated regions are different, they are specific, especially and according to Stoinev (2004), they can be solved only through the implementation of complexes of activities united in a comprehensive ecological farming system.

The physical characteristics of the soil are essential for the development of the reproductive potential of agricultural crops (Galeva and Dilkova 1970; Kuznetsova 1990; Arvidsson et al., 2003; Yankov, 2005; Van der Akker, 2006; Schjønning et al., 2020).

In soils with heavy soil texture composition (Haplic Vertisols), the physical properties are more susceptible to negative changes, necessitating timely agrotechnical intervention (Holmberg, 1985; Dimitrov & Borisova, 1996; Radford et al., 2000; Castellini et al., 2006; Grifith et al., 2013; Moreira at al., 2016) These changes require a systematic approach to adjust the physical parameters in the range of optimal values.

The Haplic Vertisols, widespread in the country, are characterized by considerable diversity in nutritional status as a result of the different fertilization systems applied. According to studies conducted by Samalieva and Nikolova (2005) on Haplic Vertisols, the recommendable content of absorbable phosphorus is 12,9-19,8 mg/100g soil and mobile potassium - 16,1-20 mg/100g in acetate-lactate extract. Ensuring optimum crop development with minimal environmental impact involves the correct determination of fertilization rate, fertilizer form, methods and timing of application (House et al., 1984; Ivanov, P., 2004; Hou et al., 2011; Nikolova et al., 2014).

By assessing basic agro-technical factors, optimal decisions can be made for good agricultural practice on soils with heavy soil texture, such as the Haplic Vertisols.

The aim of the study is to identify the changes in the physical and agrochemical status of Haplic Vertisols - that occur under the influence of the applied agro-technical measures.

Material and methods

For the achievement of the purpose in the period 2016 -2019, a field experiment, based on the block method – standard (on the long plots) in the Bozhurishte trial base of ISSAPP “N. Poushkarov”, Sofia Region, on the soil type Haplic Vertisol (according to Koinov, 1987 or WRB for soil resources) was conducted.

The field trials has a total area of 0.72 ha, includes two crop rotations of 0.30 ha (Scheme 1), each with 24 crop areas of 70 m^{-2} in size. One crop rotates the two-shelf wheat-maize crop, while the other includes other cereals with a fused surface - oats and triticale.

Scheme 1. *Crop rotation and fertilization*

Years	2016-2017	2017-2018	2018-2019
Crop rotation			
First rotation	Wheat $N_0/N_{14}P_{10}K_6$	Maize $N_0/N_{16}P_{10}$	Wheat $N_0/N_{14}P_{10}K_6$
Second rotation	Oats $N_0/N_{14}P_{10}$ K_6	Maize $N_0/N_{16}P_{10}$	Triticale $N_0/N_{14}P_{10}K_6$

Fertilization applied in crop rotation is: T_0 - no fertilization; T_1 is at a fertilizer norm according to the macroelement stock by agrochemical analysis of soil samples.

For cereals fertilization is with nitrogen, phosphorus and potassium, and for maize - without potassium. In both crop rotations, the fertilization rate is the same in order to detect changes in soil agrochemical parameters under the same conditions.

Scheme 2. *Soil tillage systems in crop rotation*

By No	Crop	Year	Soil tillage systems	
			O_1	O_2
1.	Wheat/ Oats	2016-2017	Plowing 18-20 cm	Discing 10-12cm
2.	Maize	2017-2018	Loosening 35-40cm	Plowing 28-30 cm
3.	Wheat/ Triticale	2018-2019	Plowing 15-18cm	Discing 10-12 cm

The experiments are based on a two-factor scheme of type 2x3 with four blocks (repetitions), each of which is divided into two sub-blocks corresponding to the two tillage systems (Scheme 2). One of the systems involves more intensive cultivation (O_1 variant), while the other includes a disking as a minimum soil tillage (O_2) variant.

Indicators studied:

- soil texture composition - Kachinski’s method(1958) with the pyrophosphate;
- moisture content by weight% and bulk density in g/cm^{-3} - by Kachnski's weight method, in layers 10 cm deep to 60 cm from the profile with rings of 100 cm^{-3} volume;
- total porosity of the soil - by the ratio of volume and relative mass of the soil by the formula:

$$V = \frac{d - \rho}{d} \cdot 100$$

where: V - total soil porosity in%; d - relative soil mass;

ρ is the bulk density of the soil in g/cm^{-3};

- mobile forms: nitrogen - by the method of Bremner and Kiney(1966); phosphorus - by the method of P. Ivanov(1984); potassium by the method of P. Ivanov(1984);
- reaction of soil solution (pH) - potentiometrically in water (H_2O) and potassium chloride (KCl);
- carbonates - by the method of Scheibler;(Penkov et al., 1991);
- content of organic matter in soil - according to Tyurin (Penkov et al., 1991);

Productivity is determined - the main and additional production of the cultivated crop, calculated in kg/ha;

The mathematical and statistical analysis of the experimental data was performed with the SPSS statistical program.

The experiments were carried out at the Bozhurishte experimental field of ISSAPP "N. Poushkarov" on Haplic Vertisols with a deep (about 1 m), slightly clay humus horizon. Deeper clay, dark brown, transitional horizon, which changes to a depth of about 250 cm from the soil-forming materials, follows deeper into the profile.

Table 1. *Soil Texture % to air dry condition (Haplic Vertisols)*

Horizon and depth in cm	Loss from HCl	Sum > 1	1-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	Sum <0.01
Ap 0-26	2.5	0.0	1.9	9.1	13.0	12.1	9.1	52.3	73.5
A′ 26-40	2.6	0.0	1.7	12.0	12.1	8.2	8.1	55.3	71.6
A″ 40-56	2.2	0.0	1.6	9.7	14.0	8.7	7.3	56.5	72.5
A‴ 56-85	2.2	0.0	1.4	9.7	12.4	8.3	7.3	58.7	74.3
B_k85-100	7.8	1.6	6.6	11.9	14.1	15.7	4.4	37.9	58.0
B_{ck} 100-140	8.7	1.5	3.3	9.0	15.2	8.2	10.2	43.9	62.3
C_k 140-170	8.7	0.0	7.7	13.5	14.1	6.9	7.6	41.5	56.0

The chemical properties of the soil in the experimental field vary slightly, which is a sign of soil uniformity. The humus content of the soil is medium humus (2.5% -3.8%) There is a regular and gradual decrease of humus from the surface to 120 cm depth. Total nitrogen content is low to moderate, which gradually decreases in profile depth. The total phosphorus content of the soil is poorly preserved. There is a deep leaching of carbonates from the soil profile. They are beyond the humus and transitional horizons.

The soil has a neutral to slightly alkaline reaction in the humus horizon, alkaline in the transient and strongly alkaline in the "C" horizon (Teoharov et al., 2005).

The investigation period covers years that vary in the amount and distribution of the fallen rainfall during the growing season of the crops. During the vegetation of wheat, the sum of rainfall is 346,3 mm, which is about the average for many years. Oats growth and development also proceeded at relatively good rainfall – 212,4 mm from germination to waxy maturity. During the growing season of maize - May - September, the amount of rainfall is 373,7 mm. In quantity, it is above the average for many years, but there was also a period of drought - August is only 3.8 mm.

Results and Discussion

The instantaneous moisture content of the soil found at the beginning and end of the vegetation season of wheat is influenced by the applied agricultural techniques. More significant is the influence of the type of soil tillage applied. As a result of the plowing, a higher moisture content was found in the Haplic Vertisols – 2.15% and 1.80% for the layer 20-30 cm, 1.60% and 1.80% for the layer 30-40 cm (fig. 1). For the deeper layers, the differences are statistically proven.

At the end of the growing season, permanent drought occurs after flowering. The soil moisture content recorded during this period is low. At Vertisols, the humidity is about wilting moisture17.0-19.45%, with the exception of the deepest layer 40-60 cm in the profile, where the humidity is 59-63% of MFMC. Depending on the variants of tillage, a lower content in the deep layers of 30-40 cm and 40-60 cm of the plots with applied disking was found, which is due to increased evaporation by capillary way.

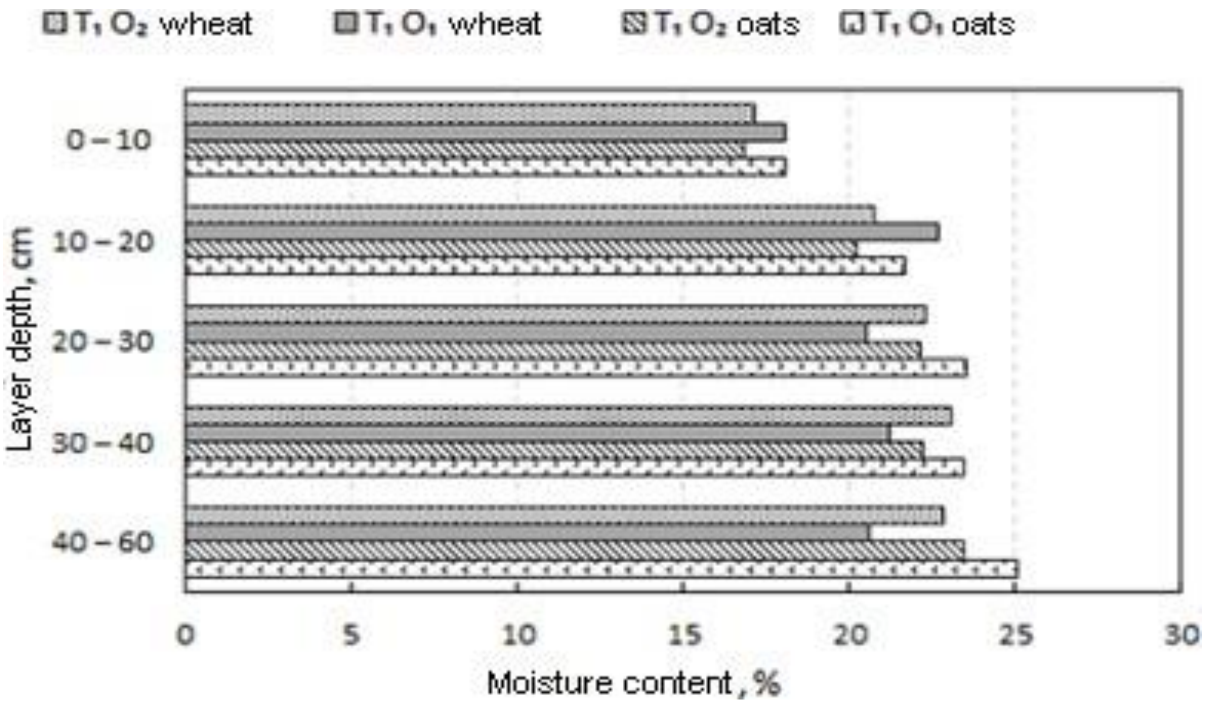
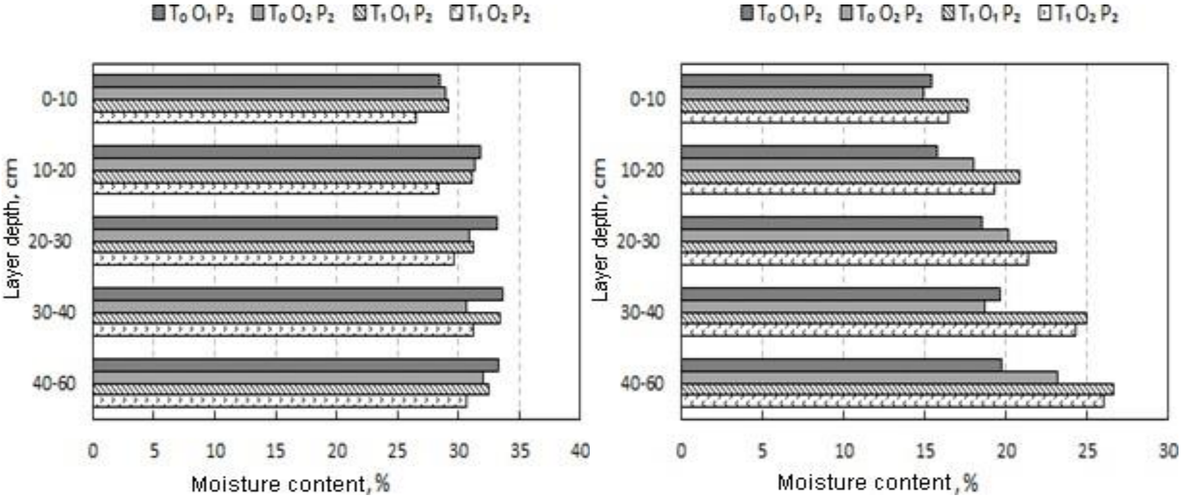


Fig. 1. *Soil moisture content in weight % - wheat and oats, 2017*

The instantaneous moisture content of the soil established at the beginning (phase "3-5 leaf") and the end of the maize vegetation season is influenced by the weather conditions and the applied agrotechnics. More significant is the influence of the type of applied tillage (fig.2,a). As a result of the basic treatment, loosening of 32-35 cm compared to 25-28 cm plowing revealed a higher moisture content of 1.41% and 2.69% for the 10-20 cm layer, respectively 2.27% and 1.64% for the 20-30 cm layer. For the deeper layers, the differences are statistically proven.



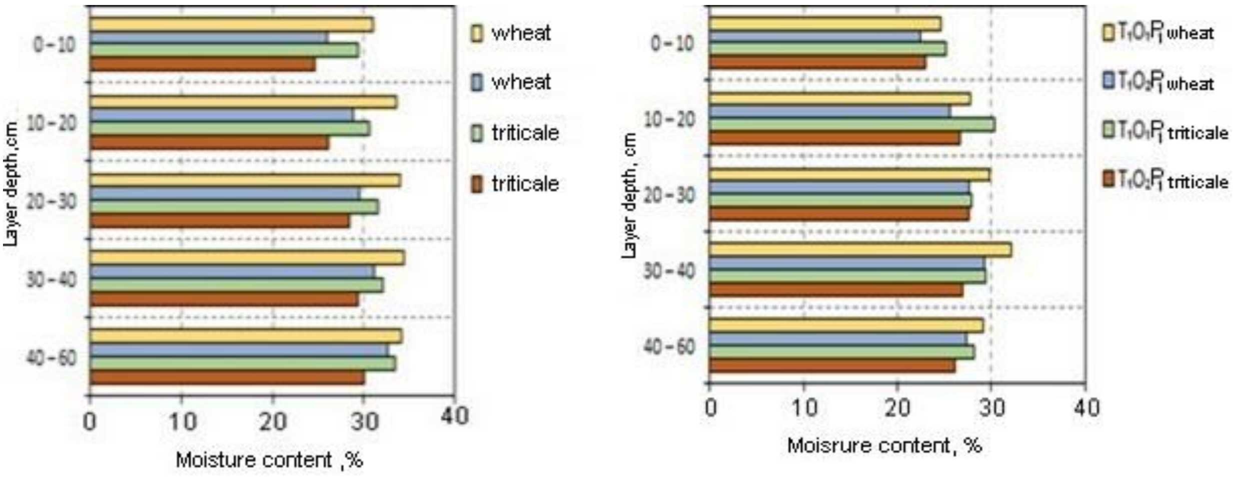
a) **b)**

Fig. 2 .*Soil moisture content in weight % - maize - 2018*
a) *phase "3-5 leaf"* ***b)*** *phase "tasseling",*

After the "tasseling" phase, a permanent drought occurred, which continued until the end of the growing season. The soil moisture content recorded during this period is low. In the Vertisols, the humidity in the plow layer is below or around wilting moisture of 14.91 – 23.11% (fig. 2b). With the exception of the deepest layer of the profile 40-60 cm, where the humidity is 50-66% of the MFMC, the soil moisture deficit is strongly manifested.

Depending on the variants of tillage, a lower content in the deep layers of 30-40 cm and 40-60 cm in the plots with zero fertilization has been found, which is due to increased evaporation due to the lower vegetation coverage of the soil surface. Unlike the initial stages of maize development, the waxy maturity phase has no established effect on the type of treatment applied.

In the wheat “breeding” phase, the humidity of the soil is 75-80% of the MFMC. Significant differences in exploration options have not been identified. In the $T_1O_1P_1$ variant the 20-40 cm layer, the moisture content was 3.16 to 3.62% higher than that found in the $T_0O_2P_1$ and $T_1O_2P_2$ variants with disking, which is due to better moisture movement during plowing (fig.3,a).This thesis is confirmed by the fact that the variants with plowing are with the highest soil moisture in the layer 30-40 cm, and those with the disking in the layer 10-30 cm. There is no established difference between fertilized and non-fertilized variants.



a) **b)**

Fig. 3. *Soil moisture content in weight%-wheat and triticale - 2019*
a) *phase “breeding”* ***b)*** *phase “class formation”*

In the “class formation” phase, the soil moisture content is much lower. In the plowing and fertilizing variant, $T_1O_1P_1$shows the biggest difference from the established in the ”breeding” phase, with the humidity in the 10-30 cm layer being over 10% lower than the previous reading. In this layer, the moisture content is on average 2.6% lower than that founded in the other variants. This finding can be explained by the soil composition in the plow layer. During plowing, the structure appears to be more friable, creating conditions for enhanced capillary rise and loss of moisture from evaporation.

In the “waxy maturity” phase, as a result of drought occurred, the soil moisture reserves are significantly reduced (fig.3,b). In the 0-10 cm surface layer, the humidity is about wilting moisture (WM). In all variants relatively balanced moisture content is established. Minor differences were found in the deepest layer 40-60 cm.

Bulk density of Haplic Vertisols

Bulk density is correlated with soil moisture. Parameter values that are within the range optimal for the development of the plant root mass are reported. An exception to this tendency is the values reported for the 30-40 cm layer in the variants with disking (fig. 4).

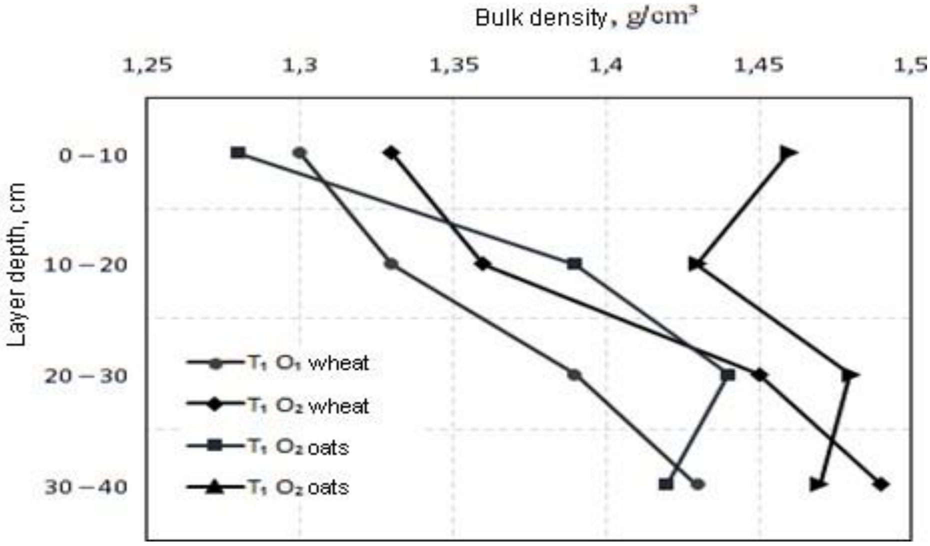
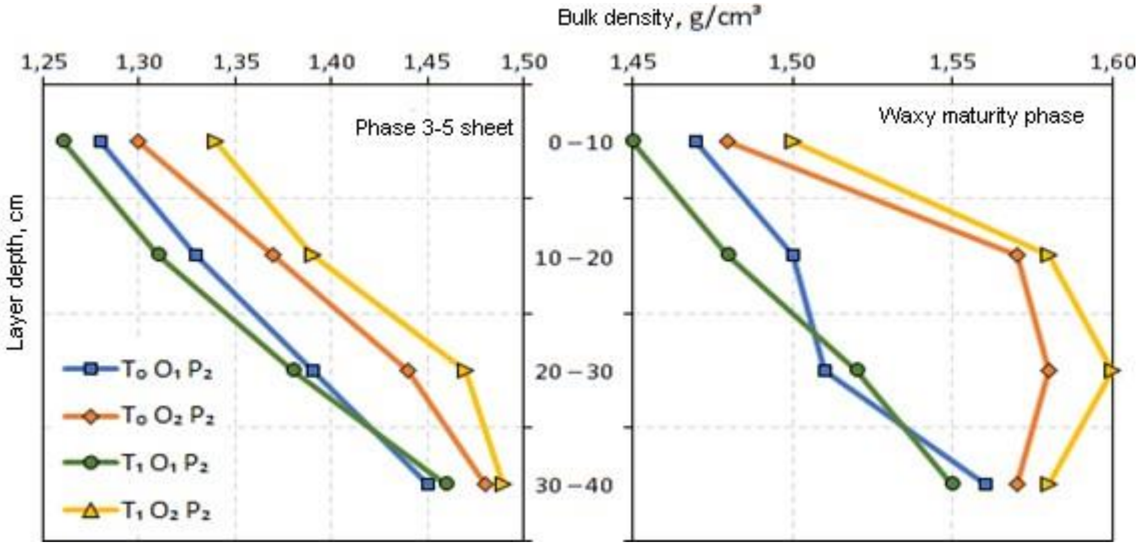


Fig. 4. *Soil bulk density in g/cm^3 – wheat and oats, 2017*

For the 30-40 cm layer, the parameter values are 0.10 g/cm^{-3} and 0.16 g/cm^{-3} higher on the plots with plowing and disking in the second crop rotation than those with the variants in the first. Although the humidity at the time of determination is about 70% of the MFMC, the values for the bulk density reach the upper limit of the optimum values (1.4 g/cm^{-3}).



a) **b)**

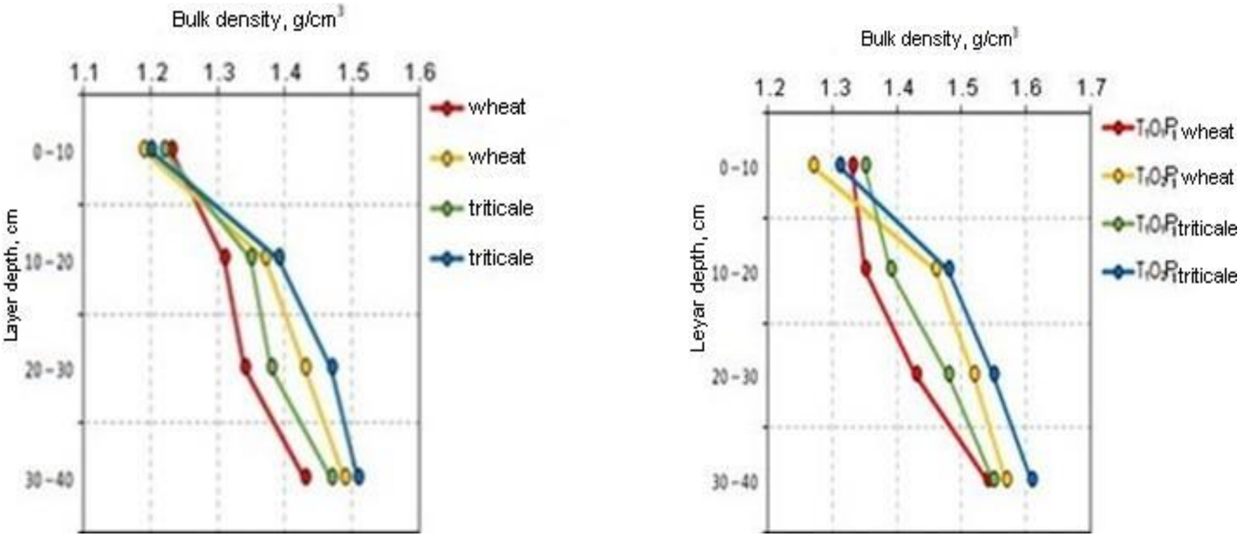
Fig. 5.*Soil bulk density in g/cm^3 – maize,* ***a)*** *"3-5 leaf" and* ***b)*** *"tasseling" phase, 2018*

At the Haplic Vertisols at the end of the growing season, the measured values for this parameter were above the equilibrium density of 1.2-1.3 g/cm^{-3}. After plowing at a depth of 15-18 cm, the soil in the 10-30 cm layer is more compacted, with a volume density of 1.56 g/cm^{-3} at a depth of 20-30 cm. The bulk density of the plots having a disking of 10-12 cm is higher than in the case of plowed variants - the difference is most significant in the layer 10-20 cm – 0.16 g/cm^{-3}.Due to the significant moisture deficit, the bulk density has high values for the entire investigated profile up to 40 cm.

In the second year, in the "3-5 leaf" phase of maize, values for the parameter, which are in the range optimal for the development of the root mass of the plants, were reported. An exception to this tendency is the values reported for the 30-40 cm layer for plowed variants.

For the 10-20 cm layer, the parameter values are with 0.04 g/cm^{-3} and 0.08 g/cm^{-3} higher on the plowed plots compared to those with a deeper loosening. Although the humidity at the time of determination is about 70% of the MFMC, the values for the bulk mass in the 20-40 cm layer reach the upper limit of the optimum values (fig. 5,a).

At the Haplic Vertisols at the end of the vegetation season, values for this parameter were measured above the equilibrium density of 1,2-1,3 g/cm^{-3}. After plowing at a depth of 25-28 cm, the soil in the 10-30 cm layer is more compacted, with a volume density of 1,60 g/cm^{-3} at a depth of 20-30 cm (fig. 5,b). The differences in the values for the bulk density of the plots with loosening of 32-35 cm compared to the variants with plowing in this layer are statistically proved. Due to the significant moisture deficit, the bulk density has high values for the entire investigated profile up to 40 cm.



a) **b)**

Fig. 6. *Soil bulk density in g/cm^3 – wheat and triticale, a) "breeding" and "class formation" phase*

In the third year, the lowest values were reported in the 0-40 cm layer in the plowed and fertilized $T_1O_1P_1$ variant in the wheat breeding phase, with differences of 0,06 g/cm^{-3} and 0,07 g/cm^{-3} for the 20-40 cm layer, and respectively are statistically proven (fig. 6,b).For the 0-30 cm tillage layer, the parameter values are up to the limits of the optimum density for this soil difference.

When considering the parameter in the grading phase, with the exception of the plowing and fertilizing variant $T_1O_1P_1$, a significant increase in the volume density was found in all 20-40 cm layers, the highest value being expressed in the $T_1O_2P_1$ variant - from 1,52 g / cm^{-3} to 1,55 g/cm^{-3}. The difference in bulk density between plowed variants must be noted. In the fertilized variant, the average difference for the studied profile 0-40 cm is 0.06 g/cm^{-3}, which is probably due to the density of the sowing and the more powerful root mass of the plants after the applied fertilization.

In the waxy maturity phase, due to the lack of moisture, the soil thickens strongly. In the 10-40 cm layer, the bulk density has values that limit the functions of the root system of wheat plants. The strongest compaction in the 10-20 cm layer in the disking and fertilizer $T_0O_2P_2$ variant is 1.73 g/cm^{-3} (fig. 6,b).

This fact, the presence of moisture only in the layer under the subsoil, confirms the established conclusion that there is a process of considerable compaction, despite the high plasticity of the clay soil aggregates.

Total porosity of Haplic Vertisols

In the first year until the wheat “tillering” phase and the occurrence of oat rotation, as a result of the good moisture content, the total porosity in the surface layer is 54.34% and 51.55%, respectively, for the area under first and second crop rotation. Then the values decrease in profile depth and in the layer 30-40 cm it is 47.79% and 43.18%. In the “waxy maturity” phase of wheat, the total soil porosity decreases by 3-5%, more significantly in the surface layers.

As a result of the good humidification in the winter-spring period, in 2018, in the phase “3-5 leaf” of maize, there is an increase in the overall porosity of the profile depth for the Vertisols compared to the “waxy maturity” phase in wheat in the 2017 (Table 2). In the O_1 system, the percentage increase is from 8.08 to 10.70%, which is most significant for the layer 0 - 10 cm (as a result of the applied treatment and winter water supply) and 30 - 40 cm (as a result of good humidity in winter). The most significant increase in the total porosity of the layer 10 - 20 cm (14.80%) is with the highest importance for the cultivation of O_2 (Table 2, b).

By reducing the water content of the soil in the “tasseling” phase, the bulk density increases, which leads to a decrease in porosity. In the O_1 tillage system, the reduction for the layer 0 - 10 cm is the lowest - only 0.12% to 3.48% for the layer 20 - 30 cm. In the O_2 system with plowing, the porosity reduction is the greatest in the 0-10 cm layer - by 5.97% and up to 1.49% for the deepest soil layer. With the increase of the moisture deficit up to the “waxy maturity” phase, the percentage of pores in soil decreased, more tangibly in the layer of 30-40 cm, which shows that the effect of the extension is lost (Table 2, c). Kercheva and Dilkova (2005) calculated the so-called "Reference" values of the bulk density that a soil would have with an optimal, critical and limiting content of drainage-aeration pores.

In the production year 2018/2019, the tendency to decrease the porosity in profile depth for both tillage systems is maintained for the Vertisols (Table 3). During the “germination” phase, a significant decrease in the overall porosity of the 10 - 40 cm layer is observed relative to the 0 - 10 cm soil layer. In the O_1 tillage system for wheat and deep loosening in the precursor, the porosity was statistically proven to be higher than in the O_2 layer 10 - 40 cm, increasing from 2.61% for 10 - 20 cm to 2.74% for 30 - 40 cm. probably the parameter values are influenced by the effect of the basic maize processing.

Table 2. *Total porosity of Haplic Vertisols in %, maize 2018*

Variance source	*Sum of sq.m.*	*Sum of sq.m.%*	*Amount of light*	*Average area*	*F-ref.*	*Level of importance*
Depth (D)	*167.165*		*3*	*55.722*	*23.390*	*.000****
Tilth (O)	*28.755*		*1*	*28.755*	*12.071*	*.001****
Crop rotation (S)	*566.684*		*1*	*566.684*	*237.879*	*.000****
*D*O*	*4.417*		*3*	*1.472*	*.618*	*.608 -*
*D*S*	*20.234*		*3*	*6.745*	*2.831*	*.054 -*
*O*S*	*.007*		*1*	*.007*	*.003*	*.957 -*
*D*O*S*	*2.000*		*3*	*.667*	*.280*	*.840 -*
Mistake	*76.232*		*32*	*2.382*		
General amount	*865.493*		*47*			

a)

Depth (cm)	*Tilth*	*Crop rot.**	*General perc. (%)*
0 – 10	*1*	*1*	*52,239*
		2	*47,925*
	2	*1*	*51,368*
		2	*46,038*
10 – 20	*1*	*1*	*50,995*
		2	*43,522*
	2	*1*	*50,373*
		2	*42,516*
20 – 30	*1*	*1*	*49,751*
		2	*41,509*
	2	*1*	*48,259*
		2	*40,000*
30 – 40	*1*	*1*	*49,751*
		2	*42,390*
	2	*1*	*46,642*
		2	*40,503*

LSD 5% =2.929
LSD 1% =3.937
LSD 0,1%=5.208

b)

Depth (cm)	*Tilth*	*Crop rot.**	*Gen.perc. (%)*
0 - 10	*1*	*1*	*50.114*
		2	*44.403*
	2	*1*	*45.398*
		2	*43.396*
10 - 20	*1*	*1*	*48.134*
		2	*44.252*
	2	*1*	*46.517*
		2	*42.874*
20 - 30	*1*	*1*	*46.269*
		2	*42.258*
	2	*1*	*44.776*
		2	*38.868*
30 - 40	*1*	*1*	*46.891*
		2	*41.258*
	2	*1*	*45.149*
		2	*39.497*

LSD 5% =2.567
LSD 1% =3.450
LSD 0,1%=4.564

c)

By the time of the "spindle making" phase (Table 3, a,b), the porosity decreases throughout the study profile, with the decrease being most sensitive in the 0 - 10 cm layer:

7.59% for O_1 and 9.08% for O_2 treatment and respectively 3.48% and 2.98% for the 30-40 cm layer.

Table 3. *Total porosity of Haplic Vertisols in %, wheat and triticale 2019*

Variance source	*Sum of sq.m.*	*Sum of sq.m.%*	*Amount of light*	*Average area*	*F-ref.*	*Level of importance*
Depth (D)	*196.394*		*3*	*65.465*	*40.313*	*.000****
Tilth (O)	*55.666*		*1*	*55.666*	*34.279*	*.000****
Crop rotation (S)	*411.139*		*1*	*411.139*	*253.178*	*.000****
*D*O*	*9.827*		*3*	*3.276*	*2.017*	*.131 -*
*D*S*	*20.140*		*3*	*6.713*	*4.134*	*.014 **
*O*S*	*5.481*		*1*	*5.481*	*3.375*	*.075 -*
*D*O*S*	*18.334*		*3*	*6.111*	*3.763*	*.020 **
Mistake	*51.965*		*32*	*1.624*		
General amount	*768.946*		*47*			

a)

Depth (cm)	*Tilth*	*Crop rot.**	*General perc. (%)*
0 - 10	*1*	*1*	*50.995*
		2	*47.403*
	2	*1*	*47.637*
		2	*46.541*
10 - 20	*1*	*1*	*48,134*
		2	*43.629*
	2	*1*	*45.274*
		2	*40.616*
20 - 30	*1*	*1*	*47.388*
		2	*43.503*
	2	*1*	*44.527*
		2	*40.610*
30 - 40	*1*	*1*	*45.517*
		2	*42.384*
	2	*1*	*44.279*
		2	*42.239*

Depth (cm)	*Tilth*	*Crop rot.**	*Gen.perc. (%)*
0 - 10	*1*	*1*	*41.542*
		2	*41.132*
	2	*1*	*45.522*
		2	*41.887*
10 - 20	*1*	*1*	*42.542*
		2	*40.126*
	2	*1*	*35.572*
		2	*38.239*
20 - 30	*1*	*1*	*41.667*
		2	*39.855*
	2	*1*	*38.930*
		2	*34.340*
30 - 40	*1*	*1*	*39.925*
		2	*37.610*
	2	*1*	*38.433*
		2	*36.723*

LSD 5% =2.188 ***b)***
LSD 1% =2.848
LSD 0.1% =3.767

LSD 5% =4.268 ***c)***
LSD 1% =5.737
LSD 0.1% =7.590

Due to the lack of soil moisture and strong soil compaction in the "waxy maturity" phase, the porosity is significantly reduced, with differences between 17.04 % and 11.19 %

for the O_1 and O_2 variants in the 0 - 10 cm and 10.08% and 8.03% for depth 30 - 40 cm. In the "waxy maturity" phase, the porosity leveling in the depth 0 - 30 cm (from 41.54 to 41.67%) is observed in the O_1 tillage system, while in the O_2 treatment it is the lowest in the 10 - 20 cm layer - only 35.57% (Table 3, c).

From the analysis of variance, it was found that, in the spring, differences were demonstrated depending on the depth of the layer, the type of cultivation applied, and between the two crop rotations. At the end of the vegetation season, the differences in layers, less than the structural condition of the soil of the two test areas - first and second crop rotations - are well proven, while differences in cultivation variants are unproven.

Influence of the studied factors on soil nutrition.

Table 4. *Agrochemical analysis of Haplic Vertisols – wheat and oats, 2017*

By №	Variants	Depth of layer cm	pH		NH_4+NO_3	P_2O_5	K_2O	Humus
			H_2O	KCl	mg/kg	mg/100 g		%
First crop rotation–wheat								
1	$T_0O_1P_1$	0-30	6.4	5.6	13.8	0.9	23.5	3.39
2	$T_0O_1P_1$	30-60	6.2	5.4	13.2	0.7	15.4	3.04
3	$T_1O_1P_1$	0-30	6.5	5.6	22.5	1.8	26.2	3.86
4	$T_1O_1P_1$	30-60	6.9	6.1	20.7	1.1	18.7	2.95
5	$T_1O_1P_2$	0-30	5.7	4.9	21.9	5.8	24.5	3.93
6	$T_1O_1P_2$	30-60	6.4	5.6	12.1	1.2	17.4	3.11
7	$T_0O_2P_2$	0-30	5.7	5.0	17.3	3.0	26.4	3.54
8	$T_0O_2P_2$	30-60	6.3	5.5	14.9	1.1	19.7	3.47
9	$T_1O_2P_1$	0-30	5.7	4.8	14.4	5.4	21.2	3.98
10	$T_1O_2P_1$	30-60	6.1	5.4	27.6	0.7	20.0	3.34
11	$T_2O_2P_2$	0-30	6.3	5.5	21.3	7.3	33.5	4.07
12	$T_2O_2P_2$	30-60	7.2	6.6	23.0	0.5	19.7	3.16
Second crop rotation–oats								
13	$T_0O_1P_1$	0-30	6.1	5.1	13.8	1.0	27.3	3.78
14	$T_0O_1P_1$	30-60	6.5	5.6	12.7	0.6	21.4	3.17
15	$T_1O_1P_1$	0-30	6.1	5.2	19.0	3.5	28.2	3.81
16	$T_1O_1P_1$	30-60	6.6	5.7	13.8	1.0	22.6	2.94
17	$T_1O_1P_2$	0-30	5.7	4.8	28.8	4.5	40.7	4.08
18	$T_2O_1P_2$	30-60	6.1	5.4	21.3	0.5	19.1	3.35
19	$T_0O_2P_2$	0-30	5.4	4.8	13.0	7.0	30.0	3.87
20	$T_0O_2P_2$	30-60	6.1	5.3	13.2	1.5	21.8	3.27
21	$T_1O_2P_1$	0-30	5.4	4.8	21.9	7.5	28.7	3.83
22	$T_1O_2P_1$	30-60	6.4	5.5	19.0	1.4	21.8	2.73
23	$T_1O_2P_2$	0-30	5.4	4.8	24.8	6.7	26.4	3.78
24	$T_1O_2P_2$	30-60	6.1	5.3	19.0	3.1	19.6	3.54

The impact of the tillage system on soil fertility is assessed by changes in nutrient content and their accessibility to plants.

Agrochemical analysis of soil samples taken during the vegetation season showed that the content of mobile forms of nitrogen was satisfactory to good in the tested area. There was a tendency for a slight increase in the content of absorbable forms of phosphorus, from low level in the beginning of the experiment, in variants with fertilization it was 2.4-7.3 mg/100 g, in the range of unsatisfactory for the layer 0-30 cm (table 4).

Table 5. *Agrochemical analysis of Haplic Vertisols – maize, 2018*

By №	Variants	Depth of layer cm	pH		$NH_{4+}NO_3$	P_2O_5	K_2O	Humus
			H_2O	KCl	mg/kg	mg/100 g		%
First crop rotation - maize								
1	$T_0O_1P_2$	0-30	6.2	5.5	21.9	0.4	23.1	3.15
2	$T_0O_1P_2$	30-60	6.6	5.8	18.0	0.2	19.6	3.37
3	$T_1O_1P_1$	0-30	6.1	5.4	38.6	2.0	27.4	3.55
4	$T_1O_1P_1$	30-60	6.6	5.8	27.1	0.3	21.3	2.99
5	$T_1O_1P_2$	0-30	5.4	4.9	30.5	3.1	27.0	4.07
6	$T_1O_1P_2$	30-60	6.6	5.4	28.2	1.0	20.5	3.74
7	$T_0O_2P_2$	0-30	6.3	5.6	23.6	0.2	22.9	3.04
8	$T_0O_2P_2$	30-60	6.5	5.8	14.9	0.5	22.8	2.72
9	$T_1O_2P_1$	0-30	6.1	5.2	36.1	1.5	32.9	3.73
10	$T_1O_2P_1$	30-60	6.6	5.8	26.5	0.2	23.5	3.28
11	$T_1O_2P_2$	0-30	6.1	5.2	29.4	1.3	30.8	3.79
12	$T_1O_2P_2$	30-60	6.3	5.6	22.5	0.2	23.7	3.35
Second crop rotation - maize								
13	$T_0O_1P_1$	0-30	6.2	5.6	23.2	1.5	23.7	3.14
14	$T_0O_1P_1$	30-60	6.2	5.6	20.2	0.2	20.4	2.70
15	$T_1O_1P_1$	0-30	6.1	5.1	25.9	2.1	26.9	3.61
16	$T_1O_1P_1$	30-60	6.1	5.3	24.8	0.2	22.9	3.36
17	$T_1O_1P_2$	0-30	6.1	5.1	28.2	4.6	33.6	4.16
18	$T_2O_1P_2$	30-60	6.1	5.3	27.1	0.9	24.2	3.72
19	$T_0O_2P_1$	0-30	6.2	5.6	20.2	0.2	27.7	4.06
20	$T_0O_2P_1$	30-60	6.7	6.0	15.2	0.2	24.0	3.13
21	$T_1O_2P_1$	0-30	6.3	5.6	23.6	3.2	24.7	3.57
22	$T_1O_2P_1$	30-60	6.4	5.8	27.6	0.4	21.4	2.83
23	$T_1O_2P_2$	0-30	6.1	5.2	28.8	3.5	28.5	4.02
24	$T_1O_2P_2$	30-60	6.1	5.3	26.5	0.3	21.5	3.85

Phosphorus is only 0.2 to 1.7 mg / 100 g of soil in the 30-60 cm bed. This low stock is explained by the fact that low fertilization rates are applied annually in order to maintain the ecological equilibrium in the soil and also to the exports with the relatively high yields of the cultivated crops. This finding is also confirmed by the reduction in the content of digestible forms of potassium, although to a lesser extent. For potassium, the reduction in content is more pronounced for the 30-60 cm layer. It is also noteworthy that during the period of using the area in Bojurishte for experimental purposes - over 25 years, the humus content decreased on average by about 0,3%.

There was a tendency for a slight increase in the content of available fraction of phosphorus, from slightly early in the experiment to 2.0-4.6 mg/100 g soil in the fertilizer variants, in the range of unsatisfactory for the layer 0-30 cm. In the 30-60 cm base layer, phosphorus is only 0.2 to 1.0 mg/100 g soil (trace state). For potassium, the reduction in content is more visible for the 30-60 cm layer, where the content is 20.5-24.2 mg/100 g of soil. It is also noteworthy that for the period of use of the area for experimental purposes, there is no significant difference in the availability of digestible forms of potassium between fertilized and non-fertilized variants.

Table 6. *Agrochemical analysis of Haplic Vertisols – wheat and triticale 2019*

By№	Variants	Depth of layer cm	pH		$NH_{4+}NO_3$	P_2O_5	K_2O	Humus
			H_2O	KCl	mg/kg	mg/100 g		%
First crop rotation – wheat								
1	$T_0O_1P_1$	0-30	6.3	5.5	12.1	0.4	25.5	3.98
2	$T_0O_1P_1$	30-60	6.5	5.6	7.9	0.2	18.4	3.76
3	$T_1O_1P_1$	0-30	6.1	5.2	15.3	5.4	23.7	3.83
4	$T_1O_1P_1$	30-60	6.2	5.4	11.5	0.7	21.5	3.37
5	$T_1O_1P_2$	0-30	6.0	4.9	14.4	2.3	24.8	3.88
6	$T_1O_1P_2$	30-60	5.9	5.1	10.3	1.5	27.7	4.23
7	$T_0O_2P_2$	0-30	6.4	5.5	12.7	0.2	30.6	3.48
8	$T_0O_2P_2$	30-60	6.5	5.7	10.2	0.2	27.7	3.44
9	$T_1O_2P_1$	0-30	5.8	4.9	15.6	8.5	22.3	3.53
10	$T_1O_2P_1$	30-60	6.2	5.7	11.5	0.2	21.1	3.45
11	$T_1O_2P_2$	0-30	5.9	4.9	17.3	4.0	27.5	3.73
12	$T_1O_2P_2$	30-60	6.6	5.6	11.5	0.5	23.7	3.13
Second crop rotation – triticale								
13	$T_0O_1P_1$	0-30	6.1	5.2	12.8	0.2	26.0	4.01
14	$T_0O_1P_1$	30-60	6.3	5.5	6.9	0.2	19.9	3.79
15	$T_1O_1P_1$	0-30	6.0	5.1	20.2	3.6	23.8	3.95
16	$T_1O_1P_1$	30-60	6.0	5.3	16.1	0.5	19.3	3.65
17	$T_1O_1P_2$	0-30	6.0	4.9	18.4	2.8	29.4	3.76
18	$T_1O_1P_2$	30-60	6.2	5.2	19.0	0.2	25.2	4.26
19	$T_0O_2P_2$	0-30	6.2	5.6	14.4	0.2	30.5	4.11
20	$T_0O_2P_2$	30-60	6.7	6.1	11.0	0.2	23.4	3.87
21	$T_1O_2P_1$	0-30	5.9	4.9	15.6	2.1	27.7	3.51
22	$T_1O_2P_1$	30-60	6.1	5.2	12.7	0.2	26.0	4.09
23	$T_1O_2P_2$	0-30	5.8	5.2	16.7	2.8	36.1	3.64
24	$T_1O_2P_2$	30-60	6.3	5.4	12.7	0.2	25.1	3.44

In the third year was established that the content of digestible forms of nitrogen was satisfactory. Compared to the data from the previous year, nitrogen was lower in all the variants tested. This indicates that much of the available nitrogen was exported with production. This assumption is confirmed by the observance of the highest values for total absorbed nitrogen for the 0-30 cm layer in the $T_1O_2P_2$ and $T_1O_2P_1$ in wheat crops – 17.3 mg/kg and 15.6 mg/kg soil respectively (table 6). In non-fertilized variants, the content of this macronutrient is slightly lower. The content of nitrogen in the 0-30 cm layer is naturally higher, with the largest difference between the two layers being found in the $T_0O_2P_2$ variant – 5.8 mg/kg soil.

In the second crop rotation of triticale cultivation, the results of agrochemical analysis are similar. A slightly higher content of assimilated nitrogen was found compared to the one in the tested area with wheat. In view of the lower grain yields obtained from the triticale, this difference in the established quantities is real.

The highest nitrogen content was reported in the $T_1O_1P_1$ variant – 20.2 mg/kg soil, for the 0-30 cm layer. For the 30-60 cm base layer, the amount of nitrogen is highest for the $T_1O_1P_2$ variant – 19.0 mg/kg soil. On the tested site with triticale, the differences in the assimilated nitrogen content between fertilized and non-fertilized variants are more pronounced.

The content of mobile forms of phosphorus is low, with traces in the subsoil 30-60 cm layer. Although the tested area is regularly fertilized with phosphorus, its amount does not increase. In the wheat area, phosphorus reaches 8.5 mg/100 g in the $T_1O_2P_1$ variant and is even lower in the other fertilized variants. In the non-fertilized variants, phosphorus is practically absent. These findings lead to the assumption that a significant portion of the phosphorus imported into the fertilizer goes into inimitable form. At the triticale tested area, the absorbed phosphorus content is even lower, although the fertilization rate is similar to that of wheat.

The content of absorbed potassium in the Vertisols is good. In the first crop rotation in wheat variants for the 0-30 cm layer, it ranges from 22.3 mg/100 g of soil ($T_1O_2P_1$ var.) to 30.6 mg/100 g of lime ($T_0O_2P_2$). Potassium is 3-4 mg/100 g less in the 30-60 cm layer. With this macronutrient, the fertilization effect is not detected, it is probably supported by the introduction of plant residues.

For triticale variants, potassium has a higher content in the 0-30 cm layer – 23.8 to 36.1 mg/100 g of soil, respectively. In the variant with the highest potassium content - $T_1O_2P_2$, the difference in the reported amount between the two test layers was the largest – 11.0 mg/100 g.

Conclusion

The study showed that soil moisture was a major limiting factor for crop development. The soil moisture content is influenced by the type of soil tillage, although the density of the weeds is also less affected by the parameter.

The bulk density of the Haplic Vertisols is inversely correlated with the soil moisture content. In the case of deep loosening of maize, its values are reduced compared to those with plowing. Lower values were found for the parameter after plowing as a pre-sowing treatment for wheat, compared to the variants with disking.

In the case of strong moisture deficiency, the bulk density values rise to the limit for the functioning of the plant root system.

The applied fertilization mainly affects the content of nitrate and ammonium nitrogen, the phosphorus forms remain with low values, it is clearly necessary to raise the norm and with a methodical approach it is necessary to achieve a sustainable level of absorbed phosphates. The content of the absorbed potassium is still satisfactory, and based on the data from the analysis, its reduction and export with the produced products are smaller.

References

Arvidsson, I., T. Keller, K. Gustafsson, 2003. Specific draught for mouldboard plough, chisel plough and disc harrow at different Water contents. Proceedings of 16th Trinnial ISTRO Conference, Brisbane, Australia, 43-48.

Bremner J. M. D. R. Keeney, 1966. Determination and Isotope-Ratio Analysis of Different Forms of Nitrogen in Soils: 3. Exchangeable Ammonium, Nitrate, and Nitrite by Extraction-Distillation Methods. Soil Science Society of America Journal p.577-582.

Castellini, M., D. Ventrella, D. De Giorgio, M. Maiorana, A. Fiore, F. Fornaro, 2006. Hydraulic properties as affected by tillage and crop residues management in a Vertisol of Southern Italy. Proceedings of 17th Triennial ISTRO Conference, Kiel, Germany, 571-578.

Chang, C., C.V. Lindwall, 1989. Effect of long-term minimum tillage practice on some physical properties of chernozemic clay loam soil. J. Soil Scence, 69, 443-449.

Dimitrov, I., M. Borissova, 2004. Features of heavy soil mechanical tillage system. Field Crops Studies, Vol. I, No 1, 121-127. (in Bulgarian)

Galeva, V., R. Dilkova 1970. Changes in the aggregate composition of some soils depending on the field humidity. Soil Science and Agrochemistry, 1,13-23. (in Bulgarian)

Grifith D., G. Steinhardt, E. Cladivko, S. Parsans (2013). Effect of tillage and rotation on agronomic performance of corn and soybean: Twenty years study on dark clay loam soil. Journal of Production Agriculture, vol. 9, No2, p. 241-248.

Hou XIII, I., W. Wiebold, F. Fritschi, 2011. Long-term tillage and crop rotation determines the mineral nutrient distributions of some elements in a Vertic Epiaqualf. Soil and Tillage Research, 112, 1, 27-35.

House, G., B. Steinner, R. Grossley, D. Odum, 1984. Nitrogen cicling in conventional and no-tillage agro ecosystems in the southern Piedmont. Journal Soil Water Conservation, 39, 3, 194-200.

Ivanov, P., 1984. New acetate – lactate method to determine the available for plants phosphorus and potassium in the soil, Soil Science and Agrochemistry, No. 3. (in Bulgarian).

Ivanov, P., 2004. Genotype characteristics of mineral nutrition. Overview. Field Crops Studies, Vol. I, No.2, 278-284. (in Bulgarian)

Karlen, D., C.Cambardella, I. Kovar, T. Colvin, 2013. Soil quality response to long-term tillage and crop rotation practices. Soil and Tillage Research, 133, 54-64.

Kercheva, M., R. Dilkova, 2005. The bulk density as an indicator of the aeration conditions of the soil. In: (ed. Dilkova, R. et al.). Proceedings of Conference “Management, Use and Conservation of Soil Resources”, 2005, p.1, 264-270.(in Bulgarian)

Kuznetsova, I., 1990. On the optimum soil density. Soil science, 5, 43-54 (in Russian).

Kachinsky N. A., 1958. Soil Texture and Microagregates Composition of Soils. Methods of their Determination. Academy of Science USSR, Moscow, p.192

Mitova T., I. Dimitrov, M. Borissova, D. Nikolova, 2002. Ipmact of conventional and conservation tillage on soil compaction and crop yield. Proceedings of Int. Conference "Soil under Global Change – a Challenge for the 21st Century, vol. II, 491-496.

Moreira W., C. Tormena, D. Karlen, A. da Silva, T. Keller, E. Betioli, 2016. Seasonal changes in soil physical properties under long-term no-tillage. Soil and Tillage Research, 160, 53-64.

Nikolova, M., P. Fixen, T. Pop, 2014. Good practices for sustainable management of crop nutrition. BMPSCN BULGARIA-(ed. Editors) Project of the International Plant Nutrition Institute, USA.

Penkov, M., A Daskalova, M. Cholakov, M. Mondeshka, M. Rizov, 1991. Soil Guide, UASG pub., Sofía, 296 p. (in Bulgarian).

Radford, B.J., B.J. Bridge, R.S. Davis, 2000. Changes in the properties of a Vertisol and responses of wheat after compaction with harvester traffic. Soil and Tillage Research, 54, 3-14.

Samalieva, A., M. Nikolova, 2005. Changes in the cost-effectiveness of maize mineral fertilization depending on the Vertisols availability of phosphorus and potassium. Soil Science, agrochemistry and ecology, № 4,3-8.(in Bulgarian).

Schjønning, P., M. Lamandé, T. Keller, R. Labouriaum, 2020. Subsoil shear strength – Measurements and prediction models based on readily available soil properties. Soil and Tillage Research, vol. 200, 104-111.

Stoynev, K., 2004. Ecological and technological aspects of modem agriculture. Ecoinnovation Ltd., Sofia. (in Bulgarian).

Teoharov, M., R. Ilieva, S. Nedyalkov, I. Atanassova, A. Grueva, B. Ruseva, 2005. Soil Guidelines. National Conference with International Participation "Management, Use and Conservation of Soil Resources", Sofia, 15 - 19 May 2005, p. 11 - 16.(in Bulgarian).

Van den Akker, J.J.H., 2006. Evaluation of soil physical quality of Dutch subsoils in two databases with some threshold values. Proceedings of 17 th ISTRO T. Conference, Kiel, Germany, 1615-1620.

Yankov, P., 2005. Influence of different soil tillage systems on crop rotation on some physical and agrochemical characteristics of slightly leached Chernozem. Abstract of Dr. Tesis, Sofia.(in Bulgarian).

Erosion Processes Due to Sprinkler Irrigation

Nelly Gadjalska, Rossitza Petrova, Snejan Bozhkov,
Viktoriya Kancheva
"N. Poushkarov" Institute of Soil Science, Agrotechnologies
and Plant Protection,
7 Shosse Bankya Str., Sofia 1331, Bulgaria
Corresponding Author: Nelly Gadjalska,, e-mail: gadjalska@abv.bg

Received: 3 October 2020 *Accepted: 14 January 2021*

Abstract

The study and analyses of erosion have shown that the main factors determining erosion processes under equal soil and climatic conditions are the intensity and structure of artificial rain and the ground slope. The main factor, artificial rain intensity, depends mainly on the soil infiltration rate. In sprinkle irrigation, the soil infiltration is preceded by interaction between the soil and the rainfall drops. Failure to observe this interaction has often caused irrigation erosion problems due to the impact drops on the soil. It has been proved that the bigger are rainfall drops and their intensity, the bigger are the damages to soil structure. The most important factors on which depends the degree of erosion are erosion resistance and slope of the soil surface. Bulgarian soils are classified in a seven-degree scale, based on $Q_{critical}$ - erosion-safety discharge.

This paper deals with the feasibility to choose irrigation equipment suitable for agricultural crops and having appropriate characteristics of the irrigation sprinklers in dependence of soil resistance and land slope. Both analytical and experimental approaches have been used in solving the problem.

Nomograms, aiming at determining the required parameters of irrigation equipment in order to prevent soil erosion, are presented. They are created on the base of experimental approaches for sprinkler equipment characteristics. The intensity and structure of the artificial rain in relation to the nozzle and the working pressure head of the sprinklers, on one side, and the soil erosion stability in relation to the difference in the land slope and rain intensity, on the other, have been studied.

The analysis of nomograms allows following conclusions about the application of three classes irrigation equipment in the erosive-safe area of land with high slope:

- High pressure sprinklers set with the appropriate blendes are to be applied to terrains of soils with medium to high degree of erosion resistance regardless of the slope. In the set with nozzles the field of application is the same as by 2-3 degree of erosion resistance, but the limit of the slope is 7 %.
- The erosion-safe area of medium pressure sprinklers application is wider as they can be used in soils of low erosion resistance (1-2 and 2) by limiting the slope to 3-5 %. For soils with moderate resistance (2-3) the slope limit is 3-7 %, and for soils with high resistance (above 3), the sprinklers are applicable on steep slopes.

Key words: irrigation, irrigation technologies, irrigation equipment, soil erosion, environment

Introduction

The most important role in the protection of the lands in irrigation systems plays the protection of the key elements of irrigation landscape, such as water, soil and environmental well-being, which determine the efficiency of irrigation and drainage. The appearance of irrigation erosion due to improper irrigation deteriorates the situation of the environment in the areas. This problem is particularly pronounced in all countries with developed irrigation network, especially where irrigated areas are on slopes.

About 34 per cent of the total irrigated areas in Bulgaria are eroded, among them 40 per cent are slightly eroded, 14 per have slight to medium degree of erosion and 28 per cent have high degree of erosion. 3.5 million hectares of the sprinkler-irrigated lands are located on sloped terrain (Rousseva, et. al., 2010). Therefore the problems related to soil conservation are of great interest.

Materials and Methods

In order to protect soil from erosion due to sprinkler irrigation is necessary to analyze and evaluate the factors, which determine the erosions process. The analysis of the research up to date indicates that in terrains with the same soil and climatic conditions the main factors, which determine the amount of irrigation erosion, are the intensity and the structure of the fed artificial rain. The slope of the terrain is of secondary influence, but spraying of crops, located on steep slopes, and raises the conditions for further deterioration of the areas.

The intensity of rain, which is a major factor for the erosion process, is directly dependent on the absorbing capacity of the soil.

If the rain intensity is greater than the absorbing capacity of the soil, in flat terrains is observed the appearance of puddles. In slope terrains under the same conditions is observed seepage. This seepage depends as well on the average intensity of the rain as on its momentary intensity, which by the high pressure sprinklers is higher as the average.

The Bulgarian soils are classified in a seven-degree scale, based on Qcritical - erosion-safety discharge (Krusteva et. al., 1990). This paper deals with the feasibility to choose suitable irrigation equipment for agricultural crops with suitable characteristics of irrigation sprinklers in dependence of the soil resistance and the land slope). Both analytical and experimental approaches have been used for solving the problem.

Nomograms for determining the required parameters of irrigation equipment in order to prevent soil erosion are presented. They are created on the base of experimental approaches for sprinkler equipment characteristics, received by author's investigations (Gadjalska, 1988), (Petrova, 1994), (Simeonova, et al., 1994a), (Simeonova, et al.,1994 b) and other researchers: (Petrov, et al., 1980), (Petkov et. al., 2007). The used data for the soil erosion stability in relation to the difference in the land slope and rain intensity were reported in the study of (Krusteva et al., 1990). The intensity and structure of the artificial rain in relation to the nozzle and the working pressure of the sprinklers on one side and the soil erosion stability in relation to the difference in the land slope and rain intensity on the other have been studied.

Results and Discussion

The nomograms are shown in (figs.1, 2 and 3).

The right side of the nomograms displays in a graphic form the relationship between the permissible average intensity of rain and the resistance class of the soil, under a relevant terrain slope. The zones of the relevant additional anti-erosion measures are given (A - competitive agriculture; B – construction of flow stopping furrows; C – replacement of usual sprinkling with pulse or periodical sprinkling). Determining factor in deciding on the nature of these measures is the slope of the terrain.

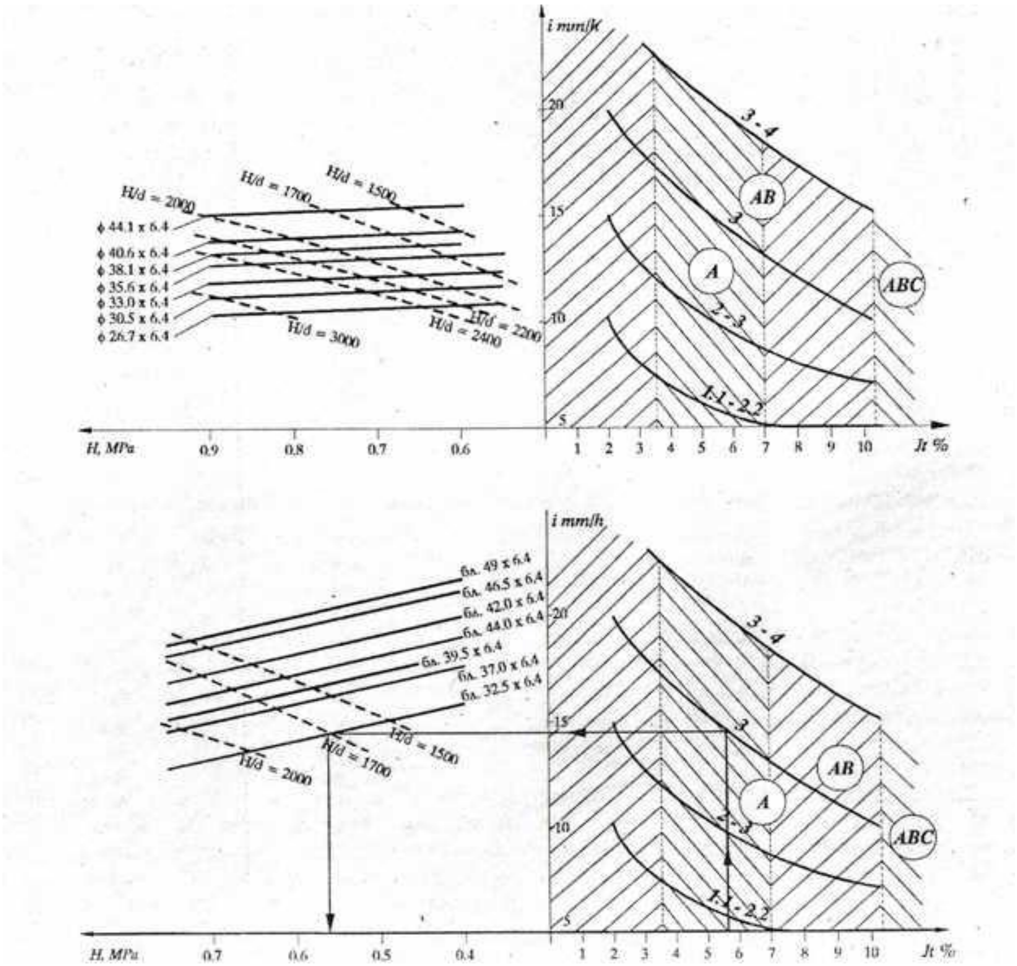


Fig. 1. *High pressure sprinklers –variants with nozzle and blende*

The left side of the nomogram shows the relationship between the technical parameters of the sprinkler head, such as working pressure and middle intensity of rain of the respective nozzle and the schedule of work.

Due to the lack of experimental data on the structure of rain for the individual sprinklers that is appreciated by criteria known H / d.

The nomogram shows that it is feasible to adjust the parameters of the sprinkler (sprinkler head and nozzle (blende)), when knowing the structure and the intensity of the artificial rain, in accordance to the erosion resistance of soil and the slope and in this way to avoid irrigation erosion.

Example of fig.1 shows that by slope of the terrain I = 5, 5 % and by third degree of erosion resistance of the soil (average sustained), the allowable average intensity of rain is 14 mm / h. By this intensity H / d = 1700 (particle size of rain drops, suitable for watering plants with good root system), sprinklers must have a blend 32.5 x6, 4 mm and to operate at pressure 0.57 MPa.

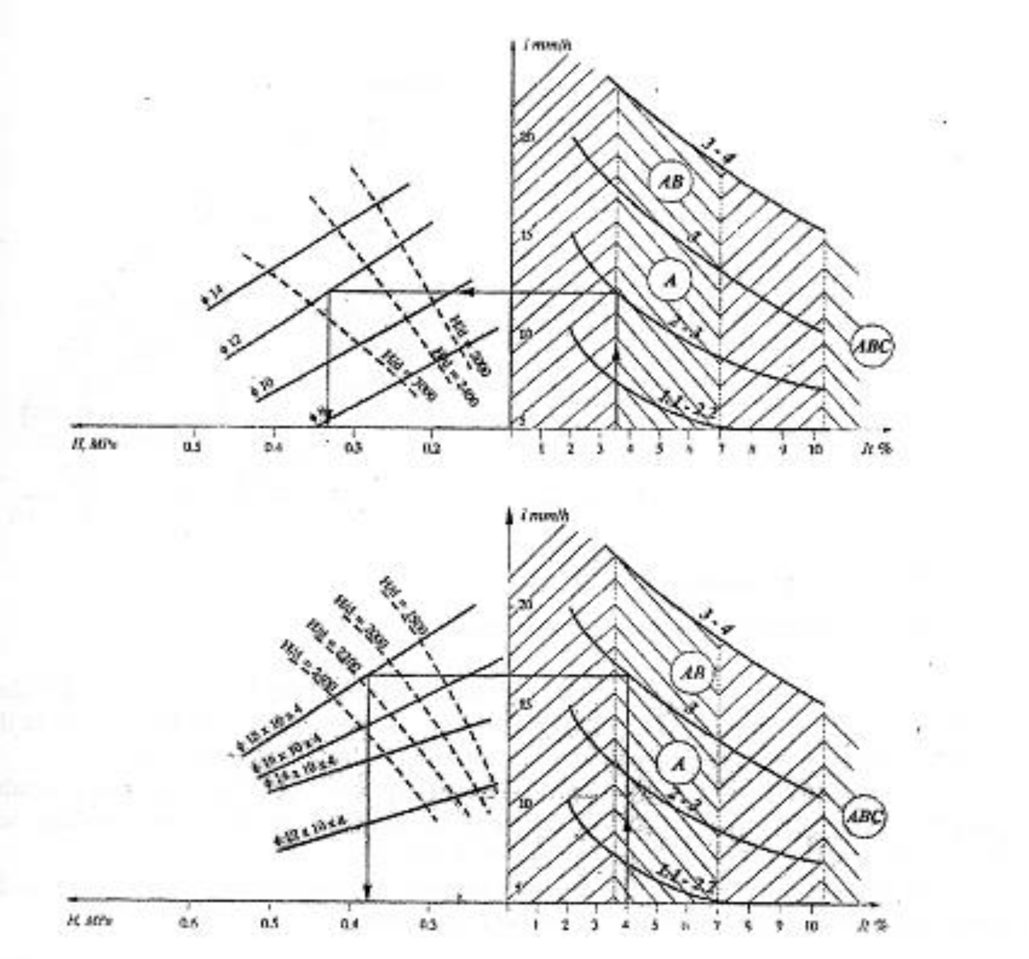


Fig. 2. *Middle pressure sprinklers*

For the middle pressure sprinklers, when the slope of the terrain is 3-5 % and soils have low erosion resistance (1-2 and 2), the allowable intensity of rain is 8 mm / h and the sprinkler must have a nozzle 16x3.5 mm. For soils with moderate resistance (2-3) and slope of the terrain 3-7 %, the allowable intensity of rain is 12-13 mm/h and a sprinkler must have a nozzle 12 mm. For soils with high resistance (above 3) the allowable intensity of rain is 16 mm / h and the sprinkler must have a nozzle 18x10x4 mm (also applicable on steep slopes).

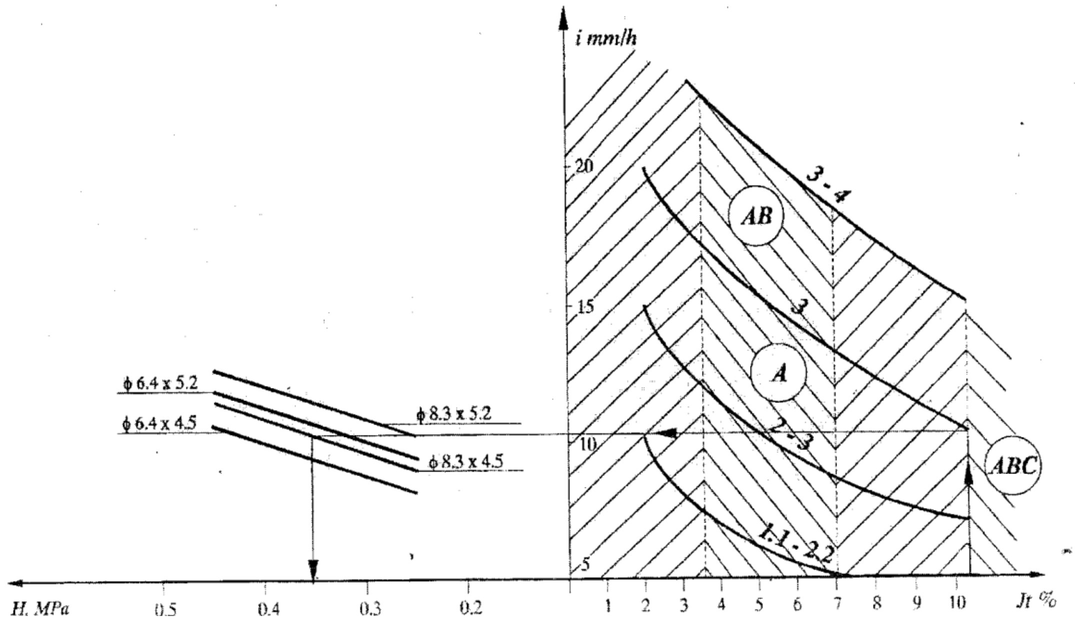


Fig. 3. *Low pressure sprinklers*

For low pressure sprinkler (fig. 3.), slope of the terrain I = 3, 5 % and 2-3 degree of erosion resistance of the soil, the allowable average intensity of rain is 12 mm / h. In this case it is recommended to use a nozzle 8.3 x 5, 2 mm in working pressure 0.42 MPa.

In the nomogram are composed all three types of sprinkler equipment produced in Bulgaria - lower, middle and high pressure sprinklers and the most used working schemas, which ensure uniformity rate of irrigation above 70 %.

In coupling the sprinklers to the installations and machinery it is necessary to do additional studies in case of the change of the working schemes.

Conclusion

The analysis of the nomogram allows following conclusions about application of the three classes of irrigation equipment for irrigation in the erosive-safe area of land with high slope:

1. High pressure sprinklers (P-90C and P-50C) set with the appropriate blende are to be applied on terrains with soils with medium and high degree of erosion resistance (from 3 to 4) regardless of the slope and also on soils with low to moderate (2-3) resistance with slopes from 3.5 to 5 %. In set with nozzles the field of application is the same as by 2-3 degree of erosion resistance, but the limit of the slope is 7 %.
2. The erosion-safe area of application of middle pressure sprinklers is wider as they can be used in soils with low erosion resistance (1-2 and 2) by limit of the slope 3-5 %. For soils with moderate resistance (2-3) the slope limit is 3-7 %, and for soils with high resistance (above 3), the sprinkler are applicable on steep slopes.
3. For low pressure sprinklers the environmentally scope varies between 5 and 9 % slope in soils with moderate resistance (2-3) and 2-3 % in soils with low resistance (1.1 to 2). For all other cases there is no limit.

In conclusion it can be said, that the proposed graphics can be used for:

- Selection of appropriate irrigation equipment in the design of irrigation fields, giving its parameters depending on soil type and slope;
- Assessment of built irrigation fields with particular consideration of the environmental performance of the equipment, used in sprinkler irrigation, before all in areas with higher slopes;

The proper complex of anti-erosion sizing parameters of irrigation equipment, in accordance to the erosion resistance of soils, allows the implementation of environmentally friendly irrigation in areas with high slope without additional investment.

References

Chehlarova-Simeonova, S., M. Neshkova, 1994a. Determination of basic sprinkler systemparameters with regard to criteria for soil protection, 17th European Conference on Irrigation and Drainage, ICID,Varna, 319-324.

Chehlarova-Simeonova, S., R. Petrova, D. Bairaktarska, S. Popov, 1994b. Using the Available sprinkler equipment under the New Economic Conditions, Proceeding of the Reasearch Institute for Irrigation, Drainage and Hydraulic Engineering, Vol.XXIV, Sofia, 86-95 (in Bulgarian).

Chehlarova-Simeonova, S., 2001. Improved expert system for chouusing of appropriate irrigation method and equipment, 19th European Conference on Irrigation and Drainage, ICID, Brno and Prague, Czech Republic.

Gadjalska, N., 1988. On some indicators of technology for sprinkling with reel-hose machnes, In support of technical progress in water management, book 2, p.18-20. (in Bulgarian)

Krusteva V., M. Neshkova, 1990. Application of anti-erosion norms in irrigation field design. Journal “Help of water management”, Col.3. (in Bulgarian)

Petrov, P., M. Harkovski, G. Gargov, 1986. Guidance for irrigation, Zemizdat, Sofia, Bulgaria. (in Bulgarian)

Petkov, Pl., R. Petrova, N. Markov, N. Gadjalska, S. Chehlarova-Simeonova, K. Karaivanov, 2007. Good irrigation practices. Avangard, Sofia, Bulgaria. (in Bulgarian)

Petrova, R.,1994. Differentiated use of Sprinkler systems with Rigid irrigation Pipelines and circle gun Sprinklers R-50 C, Proceeding of the Reasearch Institute for Irrigation, Drainage and Hydraulic Engineering, Vol.XXIV, Sofia, 80-85. (in Bulgarian).

Rousseva, Sv., L. Lozanova, D. Nekova, V. Stefanova, H. Djodjov E. Tzvetkova, I. Malinov, V. Krumov, S. Chehlarova-Simeonova, 2010. Soil Erosion Risk in Bulgaria and Recommendations for Soil Protective use of Agricultural Land, Part 1 and 2, Sofia, (in Bulgarian).

Assessment and Analysis of Designs for Fertilizer Experiments

Alexander N. Sadovski
Bulgarian Science Center of the IEAS
Corresponding Author: Alexander N. Sadovski, e-mail: bsc.ieas@yahoo.com

Received: 15 July 2020 *Accepted: 1 March 2021*

Abstract

This study aimed to present a methodology for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field with different soil types and contrasting soil-physical characteristics. Experimental designs will be used in the planning of multifactorial experiments that allow the assessment of actions and interactions of more than three factors, varying on three or more levels. Assessment of the information content of two types of designs for fertilizer trials shows their suitability for application in a basic research project. Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients - nitrogen, phosphorus, potassium, and silicon. Corresponding algorithms a given for processing of the results.

Key words: fertilizer experiments, experimental designs, statistical analysis

Introduction

Agricultural experiments have their peculiarities and differ substantially from industrial experiments. The first feature pointed out by Peregudov (1972) is that in field experiments there is a significant drift in both time and space, and the noise level is very high. As a rule, the method of steepest descent and sequential experimental design is not applicable. For this purpose, sufficiently extensive and representative experimental data obtained from single or, multiple serial homogeneous experiments are necessary.

The second feature of agricultural experiments is that they are used to study ill-defined (poorly conditioned) processes and systems whose mathematical expression is *a priori* unknown. As a result, the researcher is in a state of uncertainty and cannot make a conclusion regarding "the true model". Klevtsov and Sadovski (1981) emphasize that experimental designs must allow the simultaneous solution of two problems: the problem of discriminating between several alternative models and the problem of the optimal assessment of their parameters. Therefore such designs must contain 5 – 7 factor levels.

The objects of agricultural research are ill-defined also as a consequence of their multidimensional nature, i.e. they depend on a very large number of defining factors. And so, the third special feature of agricultural experiments is that they need to be multifactorial. Researchers often want to include 5 – 10 or more factors in their designs (Sadovski, 2018).

The starting and main point of field trials is the zero point (control). In almost all cases when quantitative factors vary, the part of interest is the positive quadrant of a hypersphere centered at the origin. This fourth feature of agricultural experiments rejects the principle of rotatability.

Many agricultural experiments detect interactions between the factors and the interactions of high order are almost always statistically not significant. This fifth feature requires that first-order interactions be evaluated.

Finally, it is well known that conducting field trials is quite difficult and if we increase the number of factors and levels, the practical implementation of factorial schemes would become impossible. Hence the sixth feature of agricultural experiments is that they need to use designs close to saturated designs. It is sensible to assume that such designs need to have at least one degree of freedom for each term in the model and one for error assessment.

Considering these six specific features of the experiment in agricultural research, and evaluation of the feasibility of the different types of multifactorial designs can be made. The study aimed to present a methodology for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field. Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients - nitrogen (N), phosphorus (P), potassium (K), and silicon (Si).

Materials and Methods

Experimental designs will be used in the planning of multifactorial experiments that allow the assessment of actions and interactions of more than three factors, varying on three or more levels. The work program of the project envisages the use of schemes of this kind (Petkova et al., 2019). The application of the following experimental designs is discussed:

A. Design, which is 1/2 replication of a 2^4 factor scheme with added control variant.

Table 1. *Design of* $1/2 \times 2^4$ *type.*

Variant	Factors			
No	A	B	C	D
1	1	1	1	1
2	2	1	1	2
3	1	2	1	2
4	2	2	1	1
5	1	1	2	1
6	2	1	2	2
7	1	2	2	2
8	2	2	2	1
9	0	0	0	0

This design will be used in conducting the field fertilizer trials with two replications. In the first 8 design variants, the triple interactions are ignored. It can assess the main effects of factors and their two-factor interactions (Davies, 1954; Nalimov & Chernova, 1965). Since this is a half-replicate, each comparison measures a pair of effects, the pairs being

(A, BCD); (B, ACD); (C, *ABD); (D, ABC); (AB, CD); (AC, BD); (AD, BC)*

Regression analysis also includes control variant No. 9. So the number of variants for this design is equal to 9. Here is a program for regression analysis of the results of the example implemented with MATLAB. The program code is applicable also with the free software GNU Octave (Eaton et al., 2020).

```
X = [ ];  # input design matrix
Y = [ ];  # input observed dependent variable
n = 18;  # number of variants with replications;
m = 4;  # number of terms in regression equation;
[n, m] = size(X);
b = (X'*X)^-1*X'*Y;            # regression coefficients
Yc = X * b;                    # calculated dependent variable
SSE = Y'*Y-b'*X'*Y;            # error sum of squares
SSR = b'*X'*Y;                 # regression sum of squares
SST = Y'*Y;                    # total sum of squares
R2 = SSR/SST;                  # coefficient of determination
sigma = sqrt(SSE/(n-m));       # standard error
F = SSR/(n*sigma^2);           # F test
pf = fpdf(F,m,n-m);            # probability of F value
T = (X'*X)^-1;                 # inverse matrix of sum of squares and products
for j = 1:m
t(j) = b(j)/(sigma*sqrt(abs(T(j,j))));
pt(j) = tpdf(t(j),n-m);
end;
t;                             # Student t
pt;                            # probability of t
```

B. Experimental design of composite type with 4 factors at 5 levels.

Table 2. *Design of type 4C1648*

Variant	Factors			
No	A	B	C	D
1	0	0	0	0
2	0	2	2	2
3	4	2	2	2
4	2	0	2	2
5	2	4	2	2
6	2	2	0	2
7	2	2	4	2
8	2	2	2	0
9	2	2	2	4
10	2	2	2	2
11	3	3	1	1
12	3	1	3	1
13	3	1	1	3
14	1	3	3	1
15	1	3	1	3
16	1	1	3	3

Concerning the six features of agricultural experiments mentioned in the introduction, a new family of multifactorial designs for the construction of dynamic yield models depending on the manageable yield-forming quantitative factors was developed (Sadovski, 1984).

The proposed experimental designs are composite and their construction can be illustrated with k = 4 number of factors. Each of the factors has 5 levels coded as 0, 1, 2, 3, and 4. The basic configuration consists of 2k star points:

(0, 2, 2, 2); (4, 2, 2, 2); (2, 0, 2, 2); (2, 4, 2, 2);
(2, 2, 0, 2); (2, 2, 4, 2); (2, 2, 2, 0); (2, 2, 2, 4);

one central point (2, 2, 2, 2) and one zero point (0, 0, 0, 0).

To evaluate the two-factor interactions of the first order, this design is combined with k(k-1)/2 points, which are part of Rechtschafner's (1967) saturated designs with two levels:

(3, 3, 1, 1); (3, 1, 3, 1); (3, 1, 1, 3);
(1, 3, 3, 1); (1, 3, 1, 3); (1, 1, 3, 3).

This configuration resembles a kite and provides degrees of freedom for estimation of the constant term, 4 linear and 4 quadratic terms, 6 interactions, and the error.

The proposed design in Table 2 will be used in conducting of greenhouse experiments. This design with a number of factors - 4 and a number of levels - 5, allows obtaining regression models in the form of a complete second-degree polynomial or other function (linear or nonlinear) with up to 15 unknown regression coefficients. ANOVA can evaluate first-order main actions and two-factor interactions.

Regression model such as second-degree polynomial

$$y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_i X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} a_{ij} X_1 X_j$$

or other functions (linear or nonlinear) of the factors $y = \varphi(X_1, X_2, ..., X_k)$ can be fitted with unknown regression coefficients whose number does not exceed (k+1)(k+1)/2.

In the case of multiple linear models, the regression analysis can easily be performed after Gram-Schmidt orthogonalization. For example, the matrix presented in Table 2 is transformed using the procedure described by Draper and Smith (1973). When the columns of the *X* matrix are almost dependent, the *X'X* matrix will be almost singular and difficulties in inversion, including large round-off errors, are likely.

As a result, the following regression equation is obtained

$$y = b_0 + \sum_{j=1}^{n} b_j z_j,$$

where z_j are orthogonal variables, linear combinations of X_j. The information matrix $Z'Z$ and its inverse $(Z'Z)^{-1}$ are diagonal.

After determination of the coefficients b_j by the formula

$$b = (Z'Z)^{-1} Z'y$$

a backward substitution is performed in the regression equation.

The corresponding estimation of the values of the dependent variable is

$$\hat{y} = Zb.$$

It should be noted that the use of the described composite designs in agricultural research is connected to the application of the two main principles of experimental planning – repetition and randomization.

The program for the regression analysis of the results of pot experiment, implemented with MATLAB differs from that for the field fertilizer trials. The first two statements differ, reflecting the size of matrix *X*. So the number of variants for this design *n* is equal to 16 and number of the terms *m* depends on the type of multiple linear regression equation. Also, the matrix *X* is transformed into matrix *Z* by orthogonalization.

```
n = 16;  # number of variants
m = 4;  # number of orthogonal polynomials
[n, m] = size(X);
Z = zeros(n, m);
R = zeros(m, m);
for j = 1:m
v = X(:,j);
for i = 1:j-1
R(i,j) = Z(:,i)'*X(:,j);
v = v - R(i,j)*Z(:,i);
end;
R(j,j) = norm(v);
Z(:,j) = v/R(j,j);
end;
b = (Z'*Z)^-1*Z'*Y;           # regression coefficients
Yc = Z * b;                   # calculated dependent variable
SSE = Y'*Y-b'*Z'*Y;           # error sum of squares
SSR = b'*Z'*Y;                # regression sum of squares
SST = Y'*Y;                   # total sum of squares
R2 = SSR/SST;                 # coefficient of determination
sigma = sqrt(SSE/(n-m));      # standard error
F = SSR/(n*sigma^2);          # F test
pf = fpdf(F,m,n);             # probability of F value
T = (Z'*Z)^-1;                # inverse matrix of sum of squares and products
for j = 1:m
t(j) = b(j)/(sigma*sqrt(abs(T(j,j))));
pt(j) = tpdf(t(j),n-m);
end;
t;                            # Student t
pt;                           # probability of t
```

The application of the presented algorithms is presented with two examples.

Results and Discussion

The mathematical and statistical analysis of the experimental results is carried out by several sequential methods. Analysis of variance (ANOVA) makes it possible to calculate the error of the arithmetic mean over the whole experiment, which is used to evaluate all possible differences between the variants tested. A useful property of ANOVA is that its estimates are consistent even with deviations from the normal (Gaussian) distribution. The next step is finding regression equations of different types - complete second-degree polynomial, polynomial with square roots, or other function (linear or nonlinear).

To illustrate the way of analysis of data obtained after the field fertilizer trials here is given as Example 1. Let start with the following experimental data. All calculations could be performed on an Excel table.

Table 3. *Example with data from Table 10.23 (Davis, 1954)*

Variant	N	P	K	Si	Yaver	I repl.	II repl.
1	-1	-1	-1	-1	107	106	108
2	1	-1	-1	1	114	113	115
3	-1	1	-1	1	122	121	123
4	1	1	-1	-1	130	129	131
5	-1	-1	1	1	106	105	107
6	1	-1	1	-1	121	120	122
7	-1	1	1	-1	120	120	120
8	1	1	1	1	132	131	133

Table 4. *Calculation of effects*

Variant	N*Y	P*Y	K*Y	N*P*K*	SIGN(N)*P*	SIGN(N)*K*	SIGN(P)*K*
1	-106	-106	-106	-106	106	106	106
2	113	-113	-113	113	-113	-113	113
3	-121	121	-121	121	-121	121	-121
4	129	129	-129	-129	129	-129	-129
5	-105	-105	105	105	105	-105	-105
6	120	-120	120	-120	-120	120	-120
7	-120	120	120	-120	-120	-120	120
8	131	131	131	131	131	131	131
1	-108	-108	-108	-108	108	108	108
2	115	-115	-115	115	-115	-115	115
3	-123	123	-123	123	-123	123	-123
4	131	131	-131	-131	131	-131	-131
5	-107	-107	107	107	107	-107	-107
6	122	-122	122	-122	-122	122	-122
7	-120	120	120	-120	-120	-120	120
8	133	133	133	133	133	133	133
Total	84	112	12	-8	-4	24	-12
Effects	21	28	3	-2	-1	6	-3

Table 5. *Analysis of variance*

Source	Total	Effects	SSQ	SSQ %	DF	F	p
N	84	21.00	441.0	33.975	2	31.500	0.0020
P	112	28.00	784.0	60.401	2	56.000	0.0008
K	12	3.00	9.0	0.693	2	0.643	0.2894
Si	-8	-2.00	4.0	0.308	2	0.286	0.5077
NP,KSi	-4	-1.00	1.0	0.077	2	0.071	0.8185
NK,PSi	24	6.00	36.0	2.773	2	2.571	0.0657
NSi,PK	-12	-3.00	9.0	0.693	2	0.643	0.2894
Factors	208	52	1284.0	98.921	14	91.714	0.0004
Error			14.0	1.079	1		
Total			1298.0	100.000	15		

The error variance is 5.408 and on this basis N, P, and interaction (NK,PSi) is significant. The regression model, which corresponds to the data of the example and includes the control variant, has the form

$Y = b_0+b_1N+b_2P+b_3NK$

The input matrix of this model is given below:

X = [1 1 1 1;1 2 1 2;1 1 2 1;1 2 2 2;1 1 1 2;1 2 1 4;1 1 2 2;1 2 2 4;1 0 0 0;
1 1 1 1;1 2 1 2;1 1 2 1;1 2 2 2;1 1 1 2;1 2 1 4;1 1 2 2;1 2 2 4;1 0 0 0].

Dependent variable is input by replications:

Y = [106 113 121 129 105 120 120 131 99 108 115 123 131 107 122 120 133 101].

n = 18; # number of variants with replications;

m = 4; # number of terms in regression equation;

Here are the results of the regression analysis from Example 1. The regression coefficients are denoted by b with corresponding t-criterion values in Table 6.

Table 6. *Regression results of Example 1.*

Coeff.	b	t	p(t)
b0	94.741	38.640	0.0000
b1	4.306	1.664	0.1012
b2	10.056	5.959	0.0000
b3	1.500	1.148	0.1997

On Table 7 are given input values of dependent variable Y, corresponding calculated by regression values Yc and their difference O-C. Estimated coefficient of multiple determination $R^2 = 0.999$ and standard error = 3.424.

Table 7. *Comparison of the input and calculated values from Example 1.*

Y	Yc	O - C
107	109.463	-2.46
114	116.019	-2.02
122	119.519	2.48
130	131.741	-1.74
106	109.463	-3.46
121	116.019	4.98
120	119.519	0.48
132	131.741	0.26
100	98.519	1.48

The design of Table 2 is applicable to multifactorial fertilizer experiments in greenhouse conditions. The vegetation experiment will be carried out in plots with 3 kg soil in three replications. Soils from the same experimental fields will be used.

To illustrate the way of analysis of data obtained from the greenhouse trials here is given as Example 2.

Table 8. *Example of experimental data.*

Variants	N	P	K	Si	I repl.	II repl.	III repl.
1	0	0	0	0	50	52	48
2	0	2	2	2	60	62	58
3	4	2	2	2	120	125	115
4	2	0	2	2	70	71	69
5	2	4	2	2	115	120	110
6	2	2	0	2	80	82	78
7	2	2	4	2	114	116	112
8	2	2	2	0	90	92	88
9	2	2	2	4	120	125	115
10	2	2	2	2	90	92	88
11	3	3	1	1	100	105	95
12	3	1	3	1	90	92	88
13	3	1	1	3	95	95	95
14	1	3	3	1	80	82	78
15	1	3	1	3	80	82	78
16	1	1	3	3	90	92	88

Two-dimensional surfaces of mean Y values in relation to N x P variants and N x Si variants are presented in Figure 1 and Figure 2.

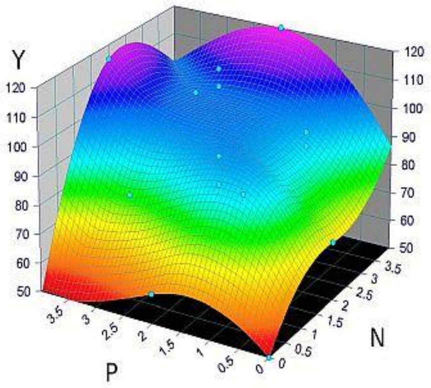


Fig. 1. *Surface N x P*

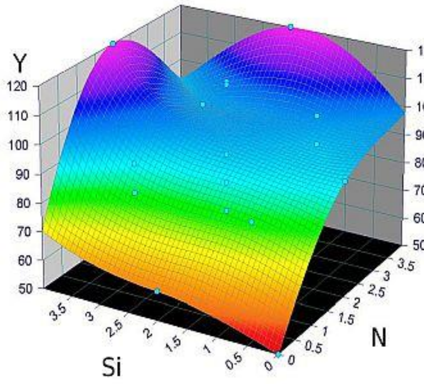


Fig. 2. *Surface N x Si*

From these figures, it can be seen with the naked eye that the increasing rates of nitrogen, phosphorus, and silicon give a significant increase in the dependent variable Y.

The statistical processing of data by means of the analysis of variance for the new design can be performed using orthogonal polynomials, which can evaluate main effects and two-factor interactions. The statistical analysis for this design is illustrated with data from Table 8

Let the dependent variable (yield) be expressed with the model

$$Y_i = \alpha_0\,\varphi_0(T_i) + \alpha_1\,\varphi_1(T_i) + \ldots + \alpha_k\,\varphi_k(T_i) + \varepsilon_i, \quad (i = 1, 2, \ldots, n),$$

where n is the number of treatment combinations; $\alpha_0, \alpha_1, \ldots, \alpha_k$ are regression coefficients; $\varphi_0, \varphi_1, \ldots, \varphi_k$ are orthogonal polynomials of the independent variables N, P, K, and Si. With T_i designates the consecutive treatment combinations in the design. It is known that the orthogonal polynomials satisfy the conditions

$$\sum_{i=1}^{n} \varphi_r(T_i).\varphi_s(T_i) = 0, \quad (r, s = 0, 1, 2, \ldots, k; r \neq s).$$

The total sum of squares is decomposed into individual components as follows (Kendall and Stuart, 1961)

$$\sum_{i=1}^{n} (Y_i - \bar{Y})^2 = \sum_{r=1}^{k} \frac{[\sum_{i=1}^{n} \varphi_r(T_i) Y_i]^2}{\sum_{i=1}^{n} \varphi_r(T_i)}.$$

Since the levels of the separate factors in the design have equidistant values: x = 0, 1, 2, 3, 4, the explicit expression of Laguerre orthogonal polynomials (Abramowitz & Stegun, 1964) up to second degree for each factor is

φ_0 = 1,
φ_1 = -x + 1,
φ_2 = (x2 - 4x + 2)/2.

The degrees of freedom for the variance of the treatments are distributed as follows: one for each component up to third-degree for the main effects of the factors and one for each of the four components for evaluation of the two-factor interactions.

When *x* is substituted with the levels of the factors N, P, K, and Si for the consecutive treatments in Table 2, the values of the orthogonal polynomials for the linear and quadratic components of the factors are obtained.

$\varphi_0 = 1$; $\varphi_1 = N_L$; $\varphi_2 = N_Q$; $\varphi_3 = P_L$; $\varphi_4 = P_Q$; $\varphi_5 = K_L$; $\varphi_6 = K_Q$; $\varphi_7 = Si_L$; $\varphi_8 = Si_Q$.

The values of the pair-wise interactions between the factors are found via term-by-term multiplication.

$\varphi_9 = N_L.P_L$; $\varphi_{10} = N_L.K_L$; $\varphi_{11} = N_L.Si_L$; $\varphi_{12} = P_L.K_L$; $\varphi_{13} = P_L.Si_L$; $\varphi_{14} = K_L.Si_{L.}$

The sums of squares of the orthogonal polynomials

$$\sum_{i=1}^{n} \varphi_r^2(T_i), \quad (r = 1, 2, ..., 21)$$

for all treatments in one replication of the design are constants and is equal to 16.

The working formulas for calculation of the sums of squares of the orthogonal components are expressed as

$$SS_{(r)} = [\sum_{i=1}^{n} \varphi_r(T_i) Y_i]^2 / \sum_{i=1}^{n} \varphi_r^2(T_i)$$

or $SS_{(r)} = \alpha_r^2 \sum_{i=1}^{n} \varphi_r^2, \quad (r = 1, 2, ..., 21).$

So we have the following values:

$SS_{(1)} = 3. -55,8974^2.16 = 149977,7328$

$SS_{(2)} = 3. -16,5067^2.16 = 13078,6101$

. . . .

$SS_{(14)} = 3. -0,7393^2.16 = 26,2337$

The multiplier 3 in the above calculations reflects the three replications of the treatments in the experiment. The results of the calculations are presented in Table 9 after combining the sums of squares of the respective components for the factors and interactions, as follows:

$SS_V = SS_N + SS_P + SS_K + SS_{Si} + SS_{NP} + SS_{NK} + SS_{NSi} + SS_{PK} + SS_{PSi} + SS_{KSi}$;

$SS_N = SS_{(1)} + SS_{(2)}$; $SS_P = SS_{(3)} + SS_{(4)}$;

$SS_K = SS_{(5)} + SS_{(6)}$; $SS_{Si} = SS_{(7)} + SS_{(8)}$;

$SS_{NP} = SS_{(9)}$; $SS_{NK} = SS_{(10)}$; $SS_{NSi} = SS_{(11)}$;

$SS_{PK} = SS_{(12)}$; $SS_{PSi} = SS_{(13)}$; $SS_{KSi} = SS_{(14)}$.

The error sum of squares is calculated by subtraction

$SS_E = SS_T - SS_V$.

The mean squares (variances) for each row in the table of the analysis of variance are obtained by dividing the sum of squares by the degrees of freedom. The experimental values of Fisher’s criterion are calculated in the usual manner through division by the error variance of the respective variance in each row. The statistical significance is verified using the F-test, as usual.

Table 9. *Analysis of variance of Example 2.*

Source of variation	SS	SS%	DF	MS	F	p%
Total	409938.00	100.00	47	-	-	-
Variants	300187.00	73.23	14	21441.93	6.4472	<0.1
N	163056.34	39.78	2	81528.17	24.5139	<0.1
P	41538.50	10.13	2	20769.25	6.2449	<1
K	22276.16	5.43	2	11138.08	3.3490	<5
Si	57429.83	14.01	2	28714.91	8.6340	<0.5
N.P	15796.41	3.85	1	15796.41	4.7497	<5
N.K	60.20	0.01	1	60.20	0.0181	-
N.Si	29.56	0.01	1	29.56	0.0089	-
P.K	1302.07	0.32	1	1302.07	0.3915	-
P.Si	3.81	0.00	1	3.81	0.0011	-
K.Si	26.23	0.01	1	26.23	0.0079	-
Error	109751.00	26.77	33	3325.79	-	-

From the analysis of variance table is evident that all main effects of nitrogen, phosphorus, potassium, and silicon are significant. Only the interaction of nitrogen and phosphorus should be taken into account.

The results of the regression analysis from Example 2 are presented in Table 10. The regression coefficients of all orthogonal polynomials are denoted by alpha with the corresponding t-criterion.

Table 10. *Regression results of Example 2.*

Term	alpha	t
Const	361.0000	0.0001
N_L	-55.8974	0.0044
N_Q	-16.5067	0.0437
P_L	-28.6324	0.0160
P_Q	-6.7496	0.1553
K_L	-21.0467	0.0284
K_Q	4.5955	0.2141
Si_L	-23.5894	0.0230
Si_Q	25.2982	0.0202
$N_L.P_L$	18.1406	0.0371
$N_L.K_L$	-1.1199	0.3094
$N_L.Si_L$	0.7848	0.3139
$P_L.K_L$	5.2083	0.1959
$P_L.Si_L$	-0.2816	0.3177
$K_L.Si_L$	-0.7393	0.3144

On Table 11 are given input values of dependent variable Y, corresponding calculated by regression values Yc and their difference O-C. Estimated coefficient of multiple determination is $R^2 = 0.999$ and standard error = 6,587.

Table 11. *Comparison of the input and calculated values from Example 2.*

Y	Yc	O - C
50	50.87	-0.87
60	57.95	2.05
120	117.60	2.40
70	69.25	0.75
115	111.75	3.25
80	77.59	2.41
114	112.61	1.39
90	84.88	5.12
120	120.82	-0.82
90	92.08	-2.08
100	104.89	-4.89
90	92.53	-2.53
95	95.08	-0.08
80	83.74	-3.74
80	81.82	-1.82
90	90.26	-0.26

The next step of the analysis is to find the optimum of the nutrient elements N, P, K, and Si.

Conclusion

A methodology is presented for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field with different soil types. Assessment of the information content of two types of designs for fertilizer trials and their suitability for application in a basic research project is presented. The objects of discussion are the design of $1/2 \times 2^4$ type and design of type ${}_4C^{16}_{48}$. The common property of both designs is that they allow studying the influence of four nutrients N, P, K, and Si. The difference is that the first allows estimation of the main effects and two-factor interactions with two levels of the nutrients. It is suitable for field fertilizer trials. The second design with a number of factors - 4 and number of levels - 5, allows obtaining regression models in the form of a complete second-degree polynomial or other function (linear or nonlinear) with up to 15 unknown regression coefficients.

Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients. Corresponding algorithms code in MATLAB is given for processing of the results. Calculations of the two examples are presented to illustrate their application. The methodology will be applied with real data from greenhouse and field experiments conducted on two experimental fields with contrasting soil types.

Acknowledgements

The author gratefully acknowledges the team members of the project. This research was performed in the framework of the Project КР-06-PN 363 funded by the Bulgarian National Science Fund.

References

Abramowitz, M., I. Stegun 1964. Handbook of mathematical functions. (Eds.). Nat. Bureau of Standards, New York.

Davies, O.L. 1954. The design and analysis of industrial experiments. Oliver and Boyd, London, pp. 654.

Draper N., G. Smith, 1973. Applied Regression Analysis. Statistika, M. (in Russian).

Eaton J.W., D. Bateman, S. Hauberg, R. Wehrbring, 2020. Free Your Numbers. GNU Octave, Free Software Foundation, Inc., Boston, MA, pp. 1094.

Kendal, M.G., A. Stuart, 1961. The advanced theory of statistics. Vol. 2, Inference and relationship. Hafner Publ. New York, pp. 684.

Klevtsov, A., A. Sadovski, 1981. Planning of multifactorial experiments and semi-empirical models on yields. Agric. Science, Sofia, XIX, 4, 108-115. (in Bulgarian).

Nalimov, V.V., N.A. Chernova, 1965. Statistical methods for planning of extremal experiments. Science, M. (in Russian).

Peregudov, V.N., 1972. On the issue of applying composite designs in field experiments with fertilizers. Agrohimiya, 6, 132-42. M. (in Russian).

Petkova, Z. B., 2019. Innovative method for optimal fertilization of agricultural crops. Work package 2: Experimental activity, Task 2.1. Project KP-06-PN 363. National Science Fund, Sofia. (in Bulgarian).

Rechtschafner, R.L., 1967. Saturated fractions of 2n and 3n factorial designs. Technometrics, 9, 569-575.

Sadovski, A., 1984. A new family of designs for agricultural experiments. Proc. Intern. Conf. Computer-based scientific research, vol. II, 580-584. Plovdiv. USWB.

Sadovski, A., 2018. Introduction to Mathematical Agronomy: Theoretical and Experimental bases. GlobeEdit, SIA OmniScriptum Publ., ISBN: 978-613-8-23895-9. pp. 302. (in Bulgarian).

The Effects of Chicken Manure Digestates on the Removal of Diesel Range Organics from Petroleum Products Polluted Soils

S. U. Oghoje*, J. E. Ukpebor, E. E. Ukpebor****
*Department of Chemistry, Faculty of Physical Sciences,
Delta State University, PMB 1, Abraka, Delta State, Nigeria.
**Department of Chemistry, Faculty of Physical Sciences,
University of Benin, PMB 1154, Benin City, Edo State Nigeria.
Corresponding Author: S. U. Oghoje, e-mail: oghojesteve@yahoo.com

Received: 12 July 2020 *Accepted: 5 March 2021*

Abstract

This study was designed to investigate the efficiency of using locally produced chicken manure digestates (CMD) as nutrient supplement for the removal of diesel range organics (DRO) from petroleum products polluted soils by land farming process. Soils samples spiked with hydrocarbon mixture (HCM), 5 % and 10 %) were treated with three rates of the CMD (0, 10 or 20 %) for a 336 days period. Soil samples were collected at day 1, 14, 28, 56, 84, 168 and 336 time interval and the samples were extracted using standard methods. The concentrations of DRO at various sampling periods were determined using a gas chromatography equipped with a flame ionization detector (GC – FID). The percentage removal of the DRO and the significance differences of the different treatments were evaluated using statistical tools.

Results obtained showed that the use of 10 and 20 % CMD led to the removal of about 50 and 58 % of the DRO respectively from the 5 % HCM polluted soils and about 41 and 35 % respectively for the 10 % HCM polluted samples at day 56 and by day 84, the percentage removal of the DRO increased to about 60, 76 and about 46, 52 respectively using the two rates of treatments; in the 5 and 10 % HCM polluted samples respectively. These values were reasonable considering the level and toxicity of HCM pollution.

Significant differences ($P < 0.05$) between the treatments and the various control samples were observed in the removal of the DRO from day 56 to 168. And at the end of 336 days, the 10 and 20 % CMD treatments enhanced the removal of about 81, 96 and 56, 67 % from the 5 and 10 % HCM polluted soils respectively but no significant differences were observed between the control and the treatment samples particularly in the 10 % HCM polluted soils at this date.

This study has revealed that: the use of chicken manure digestates could facilitate the removal of reasonable proportion of DRO in a short remediation time of 56 days from high and toxic petroleum polluted soils. The efficacy of the CMD was observed to decrease as the remediation period increased. It was also observed that 10 % CMD treatment was optimal for the removal of DRO at a short remediation period and repeated application of the nutrient supplement would be required if remediation period exceeded 56 days. Furthermore, it could be concluded that CMD is an effective inoculant and nutrient supplement during bioremediation of the DRO from toxic petroleum polluted soils. These findings are contributions to the existing data on organic stimulation for removal of diesel and by extension petroleum hydrocarbons from soils.

Key Words: chicken manure digestates, diesel range organics, bioremediation, petroleum polluted soils

Introduction

The need to clean up and reclaim petroleum polluted lands for agricultural and environmental health reasons (Okieimen and Okieimen, 2005) has been intensified in recent decades and these demands had called for several soils remediation strategies (Raskin et al., 1997; Ijah et al., 2008). Among the various soil remediation techniques developed in recent time, bioremediation techniques have gained wider acceptance and applications (Abatenh et al., 2017). This is because; the methods and materials involved are environment friendly and compactable (Mmom and Deekor 2010). Also, the technology can be localized and it is cost effective (USEPA 1994.).

Basically, the drivers of the bioremediation technology are the soil or water microbes (Bing and Kenneth, 2019). Therefore, for any bioremediation technique to work optimally there must be sufficient microbial population and diversity. Besides, the soil or water environment must be favorable for microbial growth and metabolic activity (Radwan et al., 2000, Phillip and Atlas, 2005). Naturally, there are several soil indigenous microbes that are capable of degrading soil pollutants. Soil macro and microorganisms can degrade soil contaminants in various ways including biodegradation, bioaccumulation, bio-assimilation and bio-transformation. During biodegradation the microbes use the petroleum carbon as sources of energy and end up breaking down the compounds into nontoxic compounds, water and carbon dioxide (H_2O and CO_2) (Bing and Kenneth, 2019). In bioaccumulation or bio-assimilation the soil organism such as earth worms are able to accumulate certain amount of hydrocarbon compounds into certain organs in their bodies and these compounds are metabolized or immobilized unless the organism dies while in biotransformation, certain enzymatic compounds are produces by the soil microbes which are capable of combining with the petroleum compounds making them far less bioavailable and immobile in soils. In all these ways, the population and diversity of the soil organisms as well as the soil physicochemical properties are. paramount (Radwan et al. 2000; Anoliefo 2016) The toxicity and intensity or volume of pollutants including petroleum hydrocarbons in soil is detrimental to soil microorganisms as well as the entire ecosystem (Adedokun and Ataga, 2007; Adoki and Orugbani 2007; Akpoveta et al., 2011). Consequently, a polluted land with petroleum hydrocarbon could take several years to redeem its vitality by natural attenuation. This has led several researchers exploiting various ways by which the bioremediation process can be enhanced (Adoki and Orugbani, 2007; Mmom and Deekor 2010; Akpoveta et al., 2011).

Remediation by enhanced natural attenuation (RENA) is a general term used to embrace the various approaches used to achieve the desired enhancement for the purpose of optimizing and fasten the bioremediation process. The RENA approaches or techniques include biostimulation, bioaugmentation and biofacilitation. Land farming is an example of biofacilitation for the remediation of petroleum contaminated or polluted soils. According to

USEPA, (1994) ‘Land farming is a surface-soil remediation technology for petroleum contaminated soils that reduces concentration of hydrocarbon through biodegradation to a level that is safe for human health and the environment’. It specifically involves the tilling and spreading of excavated soils to enhance the biodegradation of petroleum products (Jorgensen et al., 2000; Mmom and Deekor 2010; Silvia- Castro et al., 201; Cerqueira et al., 2014,;SPDC 2017). In some recent researches, two or more of RENA techniques were combined for more effects and efficiency (Jorgensen et al., 2000, Semple et al., 2001; Okieimen and Okieimen 2005; Oghoje et al., 2018). Such as, Phytoremediation plus phytooxidation plus land farming (Xiao-Dong et al., 2005), biostimulation and biopile (Dias et al., 2015), inoculation, composting and biopile (Gomez and Sartaj 2014), Chemo – land farming (Millioli et al., 2009); bioaugmentation plus semi-permeable reactive barrier (Xin et al., 2013) etc. Diesel is the medium distillates from crude of petroleum oils. The major constituent of diesel are the C10 – C28 and for some diesel, up to C32). It is one of the major petroleum products distributed via trucks and pipeline in Nigeria. The several usage of this product couple with its spillage in the environment either due to accidental discharge, faulty installation and storage facilities, transportation pipelines defects or sabotage has led to pollution of several agricultural land in Niger Delta region of Nigeria that calls for urgent and effective remediation protocols (Sojinu et al., 2009; Oyem and Oyem, 2013). Over the year, various inorganic and organic supplements have been used to enhance the bioremediation of diesel and other petroleum products polluted soils (Dadrasnia and Agumuthu, 2013). To the best of our knowledge, the reports on the use of chicken manure digestates (particularly generated locally) in the bioremediation of diesel from toxic petroleum products polluted soils are scanty.

Organic digestate contains high proportions of organic nitrogen (N) especially in the form of ammonium nitrogen (NH_4^+ - N) which could be available for plants and soil microbes. It also contains other macro and micro elements necessary for biochemistry and metabolism of microorganisms. Therefore, organic digestates could be a useful source of plant nutrients, an effective fertilizer for crops and microbial growth; the organic fractions of the digestate can contribute to soil organic matter turnover, thereby influencing the biological, chemical and physical properties of the soil. It is envisaged that this biostimulation supplement could use its microbial content as an additional enhancement potential for efficient and optimal degradation of the (DRO). In this study, soils spiked with HCM comprising of various percentages of diesels, BTEX and PAH were used as petroleum polluted soils to investigate the effect of CMD in optimizing the removal of the DRO. The aim is to seek a better and quicker method of degrading the DRO in the presence of other higher toxic pollutants. Specifically, the objectives of the study include to: 1) determine the physicochemical and biological properties of the chicken manure digestates 2) evaluate the rate and level of DRO removal due to the CMD stimulation 3) assess the amount of CMD required for optimal DRO removal and 4) evaluate the level of significance differences ($P < 0.05$) between the CMD treated and untreated samples

Materials and Methods

Soil, CMD and HCM sourcing

Soil collection and processing

This study was carried out at the Analytical Laboratory of the Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria, in a greenhouse environment with average day and night temperatures of 38.5^oC ±4^oC and 26.5^oC ±1.5^oC) respectively (Oghoje and Ukpebor, 2020). A composite soil was collected (at depth of 0 – 30 cm) from the University Oil Palm Estate Igue, Edo State using a spade. The bulk soil was packed into large polyethylene bags for transportation to the greenhouse for processing. The soil samples were air dried, ground and sieved through 2 mm mesh. The dried and sieved soil was stored till further use.

Chicken Manure Digestate Processing

The chicken manure digestates was locally prepared; chicken layers droppings (from a battery cage system) was collected from Sorghai Delta Farm PLC, Amuokpe, Delta State, Nigeria. Large debris (broken eggs shells, feathers, twigs, and wooden shavings etc) were removed and then the droppings were homogenized. Tap water was then added to the droppings and treated for maturity as previously reported (Oghoje and Ukpebor, 2020).

HCM procurement and Treatment

Commercially available diesel was obtained from local retailers in Benin City, Nigeria and was weathered for 3 weeks with daily stirring for about 5 minutes (Okieimen and Okieimen, 2005).

Laboratory analyses and Chemicals used

The major chemicals used in the study including; Benzene, toluene, ethyl benzene, xylene (BTEX), naphthalene, petroleum spirit and trichlorobenzene were purchased from Sigma-Aldrich, (UK). All the chemicals used for the study were of analytical grade.

Table 1. *Sequential spiking of soils with hydrocarbon mixture; HCM (adopted from Okieimen and Okieimen (Okieimen and Okieimen, 2005)).*

Remediation Indices (mg/kg soil)	5% HCM Pollution				10% HCM Pollution			
	Weeks				Weeks			
	1 (10%)	2 (20%)	3 (30%)	4 (40%)	1 (10%)	2 (20%)	3 (30%)	4 (40%)
Diesel (DROs)	4000	8000	12000	16000	8000	16000	24000	32000
Benzene	200	400	600	800	400	800	1200	1600
Toluene	200	400	600	800	400	800	1200	1600
Ethyl Benzene	200	400	600	800	400	800	1200	1600
Xylene	200	400	600	800	400	800	1200	1600
Naphthalene	200	400	600	800	400	800	1200	1600
Sub-total (mg)	5000	10000	15,000	20000	10000	20000	30000	40000
Gross total (mg)	50,000				100,000			

Source - (Oghoje et al., 2020)

Soil Spiking

The diesel, BTEX and naphthalene were dissolved in petroleum spirit to form the spiking mixture being referred to as the HCM. The soil spiking with the HCM was done sequentially, a method adopted from Okieimen and Okieimen (2005) and the samples were arranged in a Randomized Complete Block Design (RCBD) after the spiking. The total amount of the pollutants used is shown in Table 1 representing total levels of pollution with 0.8, 0.2 % or 1.6, 0.4 % being BTEX and naphthalene respectively for the 5 and 10 % HCM pollution respectively (excluding their concentrations in the diesel). That is, at day 1, 10 % of the intended HCM was added to the soil. This was increased to 20, 30 and 40 % of the intended pollution value per week. Therefore, the total pollution levels were 50 g or 100 g/kg soil for the 5 and10 % HCM pollution respectively. After the final spiking, the samples were left undisturbed for four weeks for stabilization prior to the nutrients stimulation. The floor of the greenhouse was fortified with 5 liters diesel (during the soil spiking) prior to the nutrient treatments to attract hydrocarbons utilizing microbes to the environment (Anoliefo, 2016).

Table 2, presents the description and base concentrations of the total DRO in the soil samples after stabilization and prior to nutrient supplementation for remediation.

Table 2. *Samples description and DRO concentration (conc.) at day 1 after stabilization and final treatment.*

Samples	HCM (%)	CMD (%)	CMD (g/kg soil)	Base conc. of DRO (mg/kg soil)
PSC 5	5	0	0	25302 ±244
CMD5/10	5	10	20	24507 ±241
CMD5/20	5	20	40	25317 ±257
PSC10	10	0	0	50206 ±488
CMD10/10	10	10	40	50342 ±296
CMD10/20	10	20	80	49797 ±407

Nutrient Stimulation

The CMD was used as nutrients supplement for the bioremediation of the hydrocarbons polluted soils. Appropriate amounts of the digestates were added to the samples on weekly basis for the first-four weeks according to a method adopted from Okieimen and Okieimen (2005) and were thoroughly mixed using a plastic turner to mimic landfarming. Three levels of the organic stimulation of, 0, 10 and 20 % (with respect to the level of pollution) were applied to both levels of HCM pollution. For the 5 % HCM pollution and 10 % treatment, 5 g CMD was added weekly up to the 4th week and for the 20 % treatments, 10 g of the digestates was applied weekly for the said periods while for 10 % HCM pollution the treatments were doubled (Okieimen and Okieimen 2005; Oghoje et al. 2020). Therefore, for the 5 % HCM polluted soils, the amount of CMD added to the samples were 0, 20 and 40 g/kg for PCS5, CMD5/10, and CMD5/20 respectively and for the 10 % HCM pollution, the CMD amount was doubled accordingly for PCS10, CMD10/10 and CMD10/20 (Okieimen and Okieimen 2005; Oghoje et al., 2020).

Sampling and Chemical Analysis

About 50 g of each sample was collected at the following periods: day 1 (i.e. just before the nutrient stimulation), 14, 28, 56, 84, 168, and 136. Each time the samples were properly protected and preserved for analyses at EarthQuest International Laboratory, Warri, Delta State Nigeria. The soil pH was determined at soil to water ratio of 1:2.5; the compost pH was determined at compost to water ratio of 1:5 while the digestates' pH were read directly after adequate stirring for about 5 minutes using Orion model 420A pH meter, UK, (Okalebo et al. 2002). The moisture content of the soil and the composted manures were determined by the gravimetric method, the textural analysis of the soil was by Bouyoucos hydrometer method (Okalebo et al., 2002). The Total Organic Carbon content (TOC) of the soil and the manures was determined using the Walkey-Black 1934 Method as described by Okalebo et al., (2002). Soil Organic Matter (SOM) content was calculated by multiplying the average TOC value by 1.72. Okalebo et al., (2002). The total phosphorus and total nitrogen was by Murphy and Riley method and by acid digestion-colorimetric method respectively (Okalebo et al,. 2002). Hydrocarbons analyses were done according to USEPA method 8015B using GC-FID (HP6890 USA) (USEPA 1996). The analyses for sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) were carried out as recommended by ASTM (ASTM, 1982), using a Flame Photometer (KRUSS FP8000, Germany) for K and Na and Atomic Absorption Spectrophotometer (AAS) (GBC 904AA, Australia), for Ca and Mg. And for the digestates, the physicochemical characterization as well as nutrients analyses were carried out as previously reported (Oghoje and Ukpebor, 2020).

Microbial Count

The microbial inoculation potent of the nutrients supplements were carried out according to standard methods and procedures. Heterotrophic microbes were evaluated using bacteriological agar and Rose Bengal agar for bacteria and fungi populations respectively (Anoliefo 2016). Hydrocarbon-degrading microorganisms were determined on solid noble agar plates using diesel fuel as carbon source (Margesin et al., 2000). A soil suspension was made by mixing 0.50 g of the manure in 9.50 ml of distilled water. A ten-fold serial dilution was carried out to enumerate the number of colonies. Samples were prepared in triplicates and were cultured for 8 days at 27 oC and the number of colony forming units (cfu) were counted in each sample (Colores et al., 2000). This analysis has been previously reported (Oghoje and Ukpebor 2020).

Quality Control and Statistical Analysis

Standard methods and procedures were used in samples treatments and analyses. Triplicate samples were made and the average determined. Statistical averaging, analysis of variance (ANOVA) and regression analysis were carried using appropriate statistical software. Post Hoc interpretation using Harmonic mean for the level of significance between the different nutrients stimulation with regards to DRO removal were evaluated (Oghoje and Ukpebor, 2020).

Results and Discussion

The Physicochemical characters of the Soil used for the study

The physicochemical properties of the soil used for the study has been previously reported (Oghoje and Ukpebor, 2020). The results showed that the soil was acidic with a pH of 5.42 ±0.03 which was slightly acidic in nature and typical of agricultural soils within the Niger Delta area. Similar soil pH have been reported for soils in the region (Ekebafe and Oviasogie, 2015; Falodun et al., 2015). Soil pH is a master factor in its chemistry and biochemistry. It influences the availability of nutrients and life of soil living organism

(Osodeke and Osondu, 2006; Oelkers and Valsami-Jones, 2008). Therefore, soil pH could affect soil microbial growth and bio-diversity which could hinder or reduce bioremediation of oil contaminants in soils (Oghoje and Ukpebor, 2020). For a better microbial growth and performance Dibble and Bartha (Dibble and Bartha, 1979) recommended soil pH range of 6.50 - 8.00 as optimal for hydrocarbon degradation. Similarly, Atlas (1981) reported a neutral pH value for optimal bioremediation of hydrocarbon contaminated soil. The pH of the soil used in this study is less than the recommended pH for optimal microbial activity and biodegradation. However, the results obtained showed that the CDM supplements was basic in nature and would have liming effects on the soils to which they were applied for remediation enhancement (Egwunatum, 2015; Oghoje and Ukpebor, 2020).

The soil was loamy sand having about 85, 4 and 11 % of sand, silt and clay respectively which depicts the textural properties of most agricultural soils in the rainforest of Nigeria (Iren et al., 2014; Oghoje and Ukpebor, 2020). The soil organic matter (SOM) content was relatively higher than the values of 1.18 and 1.80 % reported by Ekebafe and Oviasogie (2015) and Oshomoh and Ikhajiagbe (2015) respectively for similar oil palm estate soils. The high SOM was attributed to the regular clearing, no burning and the use of organic fertilizers which the site is known for (Oghoje and Ukpebor, 2020). Correspondingly, the macronutrients contents; N, P, and base elements were also high in comparison to similar reports (Oshomoh and Ikhajiagbe, 2015). In the absence of inorganic fertilizer application to soil, the nutrients contents relate positively and arithmetically to the organic matter content (OMC) of the soil (Oghoje and Ukpebor, 2020). The soil texture was loamy sand; therefore its water retention capacity (WRC) was relatively low having a value of about 18 % (Oghoje and Ukpebor, 2020). However, this value was within the range of WRC reported for loamy sand soils by Abdulkadir (2015) and Onwudide et al.,(2016). The soil was considered suitable for this study as it depicts the characteristics of typical agricultural soils within the Niger Delta region (of Nigeria, where oil pollution has been very challenging (Oghoje and Ukpebor, 2020).

Physicochemical, Nutrient and Microbial Potential of the CMD Supplement

In this study, the chicken layers droppings were locally fermented to generate digestate under certain abiotic conditions. The results showed that the total nutrient nitrogen for the CMD was about 1,422 mg/l while the available phosphorus was 958 mg/l as previously reported (Oghoje and Ukpebor, 2020). It has slightly alkaline pH value, 7.58 and TOC values of 5.02 %. The alkaline nature of the digestate would be favorable for bioremediation since the pH falls within pH range of 6.50 – 8.00, which is the range favorable for microbial growth and bioremediation activities (Atlas, 1981; Bossert and Bartha, 1984). Furthermore, the digestate has the least value of available phosphorus which means it would have less eutrophication tendency and be more environmental friendly compared to the other form of nutrient stimulation (Oghoje et al., 2020).

Beside the favorable physicochemical properties and good nutrient potential of the CMD, it contained adequate hydrocarbons degrading microbes. Our previous results showed that the total hydrocarbons degrading bacteria (THUB) and fungi (THUF) were as much as 1.6×10^4 and 1.3×10^4 cfu respectively. Similarly, the total heterotrophic bacteria and fungi were 1.5×10^4 and 1.4×10^4 cfu respectively (Oghoje and Ukpebor, 2020). This implies that the CMD has both nutrient supplementing and microbial inoculating potentials. It has been asserted that the major drivers of soil contaminants or pollutants bio-decomposition processes are the microbes which are mainly the bacteria and the fungi (Molina-Barahona et al. 2004; Oghoje et al. 2020). The heterotrophic bacteria and fungi are capable of feeding and oxidizing all available organic carbon source but they have preference for non-hydrocarbons substances which have potentials for easy oxidation. In others words, the heterotrophic bacteria or fungi would only go for the hydrocarbons in absence or deficiency of other organic carbon sources (Oghoje et al., 2020). But, the HUB and HUF are often attracted to hydrocarbon pollutants in soils as a result of their affinity for hydrocarbon (Akoachere et al., 2008; Okoro, 2010; Anoliefo, 2016). This behavior was observed weeks after the soil spiking.

A drastic increase in the microbial content of the polluted soils prior to the nutrient supplementation with the CMD was observed. Therefore, in a diesel or hydrocarbon polluted soils, the HUB and HUF would increase in population and diversity (provided the level of pollution is not high enough to be detrimental to their growth) and they play the major role in the bioremediation of such contaminants (Oghoje and Ukpebor, 2020). Consequently, in several bio-augmentation studies, the hydrocarbon utilizing microbes were isolated, cultured and introduced into the oil polluted environment to boost their population for optimal bioremediation (Abdulsalam and Omale, 2009; Anoliefo, 2016). For instance, the THUB and THUF of the unpolluted soil (UPS) were 3.0×10^3 and 5.0×10^3cfu respectively but were increased to 7.0×10^3 and 1.2×10^4 cfu respectively in HCM polluted soil (PSC). Similarly, the THB and THF in the UPS were 7.0×10^3 and 1.2×10^4 cfu respectively but the values increased to 1.4×10^4 and 1.5×10^4 cfu in the oil polluted soils (PCS) respectively. Also the values of all the microbes were higher in the CMD supplements soils than their corresponding values in both the UPS and PSC with the values 1.5×10^4, 1.6×10^4, 1.4×10^4 and 1.3×10^4 cfu for the THB, THUB, THF and THUF respectively.

Therefore, the manure digestates should have higher bioremediation potentials as compared to the other sources of nutrients stimulation as observed in previous studies (Oghoje and Ukpebor, 2020). The presences of HUB and HUF in similar organic wastes have been reported by previous researchers (Adekunle, 2011). These findings have indicated that chicken manures digestates could be better as alternative nutrients supplement and microbial inoculants in land farming of petroleum hydrocarbons polluted soils.

Removal of the DRO from the HCM polluted soils

The effects of three levels of CMD stimulations on the removal of DRO from soils polluted by petroleum products mixture (HCM) was determined using the analyses of total diesel range organics (TDRO) as the remediation indices. The results showed that for the 5 % HCM pollution treated with 10 (i.e. CMD5/10) or 20 % (i.e. CMD5/20) of CMD, the base concentration of the DRO were about 24507 and 25317 mg/kg soil respectively while for the 10 % HCM pollution, the total DRO concentration were 50342 mg/kg for those treated with 10 % CMD (CMD10/10) and about 49797 mg/kg for the soils treated with 20 % CMD (CMD10/20). The base concentrations of the control soils were similar with values of 25302 and 50206 mg/kg for the 5 and 10 % HCM pollution respectively (Table 2).

The percentage removal of the DRO as remediation proceeded is presented in figure 1. A general increase in the percentage removal of the DRO in both nutrients stimulated and un-stimulated samples were observed. Figure 1 showed, that the percentages remediation of the

DRO in the soils was minimal in the first two weeks especially with the untreated and the 10 % HCM polluted soils. For instance, the DRO in PCS5, PSC10, CMD10/10 and CMD10/20 were reduced by about 3.5, 0.53, 3.75 and 3.32 % respectively at day14

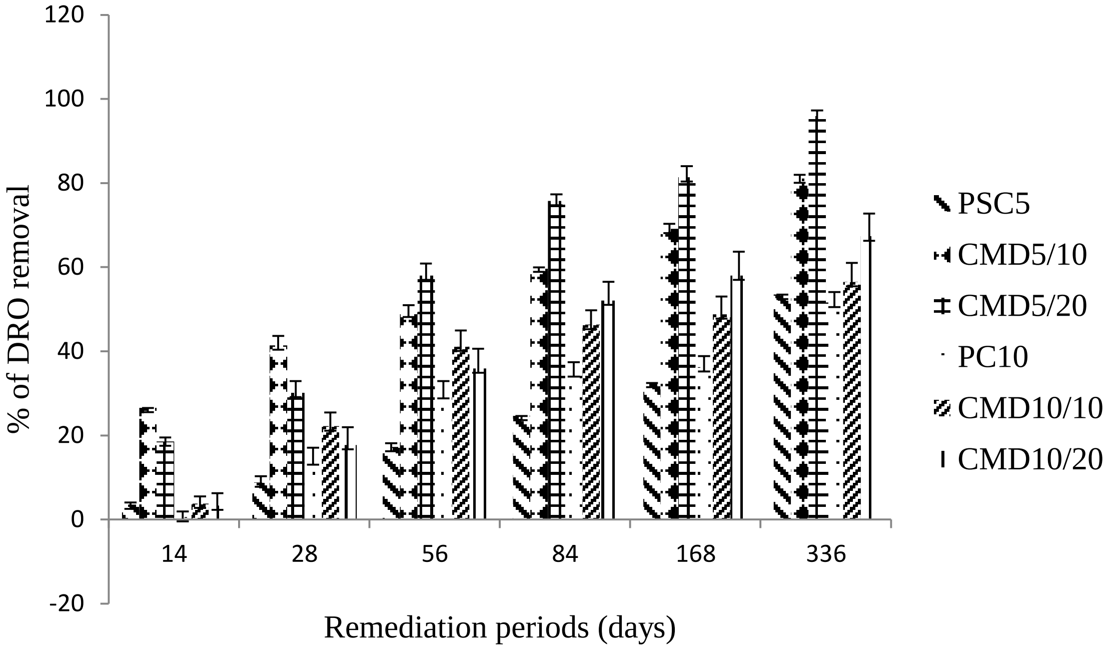


Fig. 1. *Percentage removal of DRO from the HCM polluted soils*

It has been reported that the rate and degree of bioremediation is determined by many factors including the prevailing chemical (such as pH and pollutants' concentrations), physical (moisture and temperature and biotic (microbial population and diversity) conditions (Amadi et al., 1993; Adoki and Orugbani, 2007; Mmom and Deekor, 2010). Therefore, the low remediation values at the early weeks of the process may be attributed to the high volume and toxic petroleum products such as the BTEX and PAHs present in the HCM particularly in the 10 % HCM polluted samples which could be inimical to microbial activities as reported by Adekunle (Adekunle 2011). Besides, microbes need time to acclimatize to major changes in the soil environment (Okieimen and Okieimen, 2005; Mmom and Deekor, 2010, Adekunle, 2011). However, for the 5 % HCM pollution organic nutrient stimulated samples, the values of the DRO removal were relatively higher when compared to those of the controls (PSC5 and PSC10)) and the 10 % HCM polluted samples (i.e. CMD10/10 and CMD10/20).with CMD5/10 and CMD5/20 having DRO removal values of about 27, and 19 % respectively at day 14. This improvement may be attributed not only to the organic nutrient contents of these treated samples but the microbial inoculation potentials of the organic digestates used in their stimulation. After about three months, precisely at day 84, about 60 and 76 % of the DRO were degraded from the samples treated with CMD at 10 (i.e. CMD5/10) and 20 % (i.e. CMD5/20) respectively and about 46 and 52 % for the 10 % HCM polluted samples stimulated with 10 (C10/10) and 20 % CMD. (C10/20) respectively. For the control soils, PSC5 and PSC10, the percentage DRO removal were quite low when compared to the nutrient stimulated samples with corresponding percentage removal values of 25 and 35 % respectively at the mentioned day. The results obtained from the nutrients stimulation using 10 or 20 % manure digestates were in close range with the values of 71 and 84 % DRO removal from diesel polluted soils reported by Jorgensen et al.,(2000), and Van Gestel et al., (2001), who used spruce bark and food waste for composting diesel polluted soils respectively.

These results conformed to several reports on organic stimulation of oil polluted soils. However, other environmental determinants may lead to wide variation in the values of remediation obtained from similar organic composting (Jorgensen et al., 2000; Van Gestel et al. 2001; Okieimen and Okieimen 2005; Oghoje and Ukpebor, 2020). It was observed that at short duration the use of 10 % CMD gave better remediation of the DRO as compared to the use of 20 % of the nutrient supplement. For instance at day 14, 10 % CMD enhanced the removal of about 27 % as against the 20 % CMD treatment which degraded just about 19 % of the DRO from the 5 % HCM polluted samples at this same period of remediation. Similarly, for the 10 % HCM pollution, 10 and 20 % CMD treatments led to DRO removal valued about 4 and 3 % respectively at day 14. However, beyond day 28, the 20 % CMD stimulation led to better DRO removal in both 5 and 10 % HCM polluted soils. It had earlier been documented that bioremediation required certain amount of nutrient stimulation and that the rate and/or degree of soil contaminants bio-removal are not always proportional to the amount of nutrient supplied (Okieimen and Okieimen 2005, Chorom et al. 2010, Oghoje and Ukpebor 2020). For the 5 % HCM pollution, the use of 10 % CMD was adequate at shorter periods of remediation. Okieimen and Okieimen (2005) also found the use of 10 % of rubber processing sludge (RPS) and Poultry droppings to be adequate for optimal biodegradation of the aliphatic hydrocarbons at 5 % petroleum pollution level. But for longer period of remediation particularly with high levels of petroleum pollution 20 % or repeat application of 10 % CMD would be ideal.

It was observed that the percentage of DRO degradation in all the samples up to day 56 were all less than 50 % except for the CMD5/20 samples with average value of 58 %. Therefore, it can be said that at these periods, the samples were still too toxic to support optimal microbial activities. However, reasonable percentages of DRO degradation were recorded in the samples after day 56. For example, in the CMD 5/10 and CMD5/20, the percentages of DRO removed were, 60, 69, 81 and 76, 81 and 96 % respectively at days 84, 168 and 336 respectively. Similarly, for the CMD10/10 and CMD10/20 the DRO degraded values were about, 46, 49, 56 and 52, 58 and 67 % respectively at days 84, 168 and 336 respectively. The untreated samples gave the least percentage removal of DRO as expected (in each level of pollution) when compared to their counterpart stimulated samples. It was also observed that the proportion percentage removal of the DRO were higher at the early bioremediation intervals. Figure 2 presents the percentage removal of the DRO at the remediation intervals. The results showed that for the CMD5/10 and CMD5/20 samples, greater amounts of DRO were degraded between day 0 and 14 in the 5 % HCM polluted samples. But for the 10 % HCM polluted samples greater amounts of DRO was removed in CMD10/10 and CMD10/20 samples between days 28 and 56. For instance between days 28 and 56, about 28, 19 and 18 % of DRO were degraded in the CMD5/20, CMD10/10 and

CMD10/20 respectively as against 18, 5 and 16 % degradation in these samples respectively between days 56 and 84 (Figure 2).

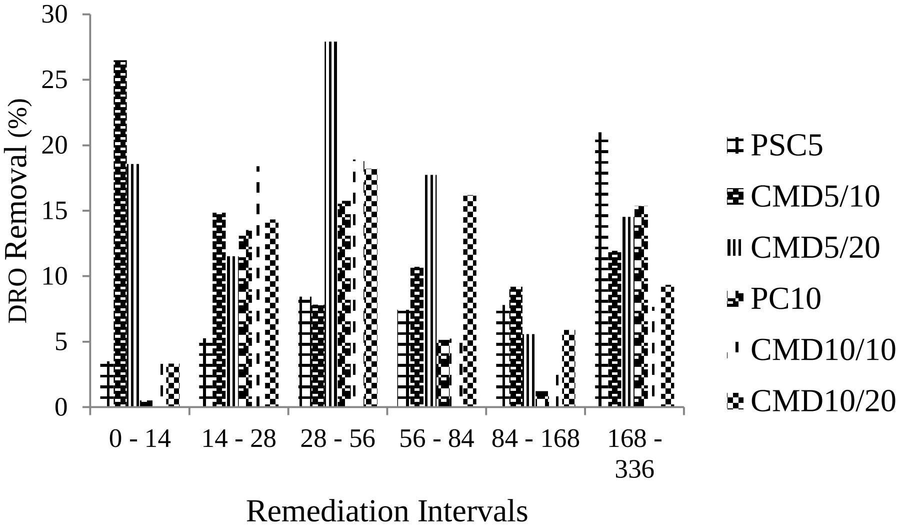


Fig. 2. *Percentage amount of DRO removed at the remediation intervals*

Similar observation was made by Okieimen and Okieimen (2005). The researchers reported higher values of degradation of saturated alkanes (DRO) at the middle phase of bioremediation of crude oil polluted soils using poultry dropping and rubber processing sludge for stimulation. This observation may be attributed to relatively ease of breaking down of the aliphatic alkanes in comparison to other families of hydrocarbons such as the aromatics. The dynamism in microbial growth, diversity and metabolisms may also be another contributing factor as well as the nutrient availability which may be favored at the early phase of the bioremediation protocol.

Adequate nutrients in the soils facilitate microbial population and diversity and consequentially increase oil degradation (Van Gestel et al., 2001). As the nutrient content of the soil depletes coupled with already reduced level of HCM pollution, the smaller amount of DRO degradation in the CMD5/10, CMD10/10 and CMD10/20 samples could be envisaged. It has been emphasized that soil microbes in oil polluted need sufficient time for acclimatization as well as adequate nutrients and favorable environmental conditions for optimal biodegradation of soil pollutants concerned (Okieimen and Okieimen, 2005; Chorom et al., 2010, Oghoje and Ukpebor, 2020). These may be the reasons why the untreated samples PSC5 and PSC10 (i.e. the controls) have their higher proportion of DRO removal, about 21 and 16 % respectively between days 168 and 336.

Significance effects of CMD on DRO removal from HCM polluted soils

It is necessary to evaluate if the treatments significantly removed these hydrocarbons with regards to the control samples. Tables 3a and b presents statistical Post Hoc analysis of percentage DRO degradation at day 84 (3 months). The results showed that there was significant difference ($P < 0.05$) between the control (PSC5) and all the nutrient stimulated samples. There was also such significance difference between the two nutrient treatments in the 5 % HCM polluted soils (Table 3a). But for the 10 % HCM polluted samples, no significant difference between percentage DRO degradation in the 10 (i.e. CMD10/20) and 20 % (i.e. CMD10/20) CMD treatments though the CMD10/20 gave higher arithmetic value of DRO removal at Day 84 than CMD10/10 (Figure 1). However, both treatments led to DRO removal significantly difference from that of the corresponding control samples (Table 3b) at day 84. It should be noted that treatments whose harmonic means appeared in the same column in the tables had no Significance differences.

Table 3a. *Post Hoc interpretation of DRO percentage removal from 5 % HCM polluted soils at day 84.*

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
PSC5	3	24.650				
CLD5/10	3				59.910	
CLD5/20	3					75.750
Sig.		0.080	0.360	0.950	1.000	1.000
Means for groups in homogeneous subsets are displayed						
Harmonic Mean Sample Size = 3.000						

Table 3b. *Post Hoc interpretation of DRO percentage removal from 10 % HCM polluted soils at day 84.*

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
PSC10	2		39.825		
CMD10/10	2			71.405	71.405
CMD10/20	2				74.385
Sig.		1.00	1.000	0.070	0.050
Means for groups in homogeneous subsets are displayed.					
Harmonic Mean Sample Size = 2.000.					

Table 4a. *Post Hoc interpretation of DRO percentage removal from 5 % HCM polluted soils at day 168*

Treatment	N	Subset for alpha = 0.05		
		1	2	3
PSC5	2	41.665		
CMD510	2	52.145		
CMD520	2			79.345
Sig.		0.620	1.000	0.190
Means for groups in homogeneous subsets are displayed.				
Harmonic Mean Sample Size = 2.000.				

Table 4b. *Post Hoc interpretation of DRO percentage removal from 10 % HCM polluted soils at day 168*

Treatment	N	Subset for alpha = 0.05	
		1	2
PSC10	4	38.463	
CMD1010	4	55.118	55.118
CMD1020	4		64.693
Sig.		0.380	0.060
Means for groups in homogeneous subsets are displayed.			
Harmonic Mean Sample Size = 4.000.			

Similarly, at day 168 (6 months), significance differences were observed in the percentage removal of the DRO between the control and the nutrient stimulated samples and between the two rates of nutrients stimulation (I.e. CMD5/10 and CMD5/20) for the 5 % HCM polluted samples (Table 4a). But in the 10 % HCM polluted samples; there was no significance difference between the control samples and the 10 % nutrient stimulated samples. Also there was no significance difference between the two nutrients stimulated samples except the observed significance difference between the control and the 20 % nutrient stimulated samples (Table 4b).

Similar trends were observed at day 336 in which there were significance differences between the percentage removal of DRO in the control samples and the two levels of nutrient stimulation and between the two nutrient stimulation levels (i.e. CMD5/10 and CMD5/20) in terms of the percentage removal of DRO at this period (Table 5a). But for the 10 % HCM polluted samples, there were no such significance differences between the control samples (PSC10) and the nutrient simulated samples (Table 5b).

Table 5a. *Post Hoc interpretation of DROs percentage removal from 5 % HCM polluted soils at day336*

Treatment	N	Subset for alpha = 0.05		
		1	2	3
PSC5	2	61.950		
CMD5/10	2		79.635	
CMD5/20	2			93.985
Sig.		0.08	0.39	1.00
Means for groups in homogeneous subsets are displayed.				
Harmonic Mean Sample Size = 2.000.				

Table 5b. *Post Hoc interpretation of DROs percentage removal from 10 % HCM polluted soils at day 336*

Treatment	N	Subset for alpha = 0.05
		1
PSC	4	55.610
CLD10	4	63.383
CLD20	4	75.433
Sig.		0.090
Means for groups in homogeneous subsets are displayed.		
Harmonic Mean Sample Size = 4.000.		

This implies that at this long period of remediation the two levels of treatments no longer produce significant effects in terms of percentage removal of the DRO even though their arithmetic percentage removal of the contaminants were higher than that of the control in the order of; CMD10/20 > CMD10/10 > PSC10 (Figure 1).

The observation may be attributed to gross depletion in the nutrient of the CMD as the bioremediation period prolong with a consequential drop in microbial population and metabolism and reduction in the degradation of the DRO (Figure 2). The results obtained from this study showed that at different stages of the remediation up to a throughput of 56, the use of 10 % CMD would be sufficient but beyond this period, the treatment no longer has significant effects on DRO removal from the HCM polluted soils particularly for the higher level of the petroleum products pollution. The use of 20 % of the CMD treatment produced significant removal of the DRO to about 168 days (6 months) even in the 10 % HCM polluted samples but as the remediation was extended to day 336, its nutrient supplement and inoculates effects became minimal. In other words, there would be drastic drop in the nutrient supply as well as gross drop in the microbial population and diversity due to the use of the manure if the bioremediation period is extended beyond days 84 or 168. Similarly, Okieimen and Okieimen (2005) reported the drop in the bioremediation potentials and effectiveness of rubber processing sludge (used for the enhancement of bioremediation of crude oil polluted soil) as the period of remediation prolongs. The researchers observed that 10 % of this organic supplement could produce optimal effects up to day 84.

The higher remediation effect of 20 % CMD treatment in comparison to the 10 % treatment may be attributed to its higher value or volume of microbes and water. In favorable conditions, the magnitude of bioremediation of soil pollutants could be directly correlated with the values of microbial population and diversity. Soil water is another important determinant of microbial growth and metabolic activities. Hence, it has direct effects on the degree and rate of pollutants degradation in soils (Molina-Barahona et al., 2004). Specifically, Molina-Barahona et al., (2004) reported 30 % soil moisture content for optimal biodegradation of hydrocarbons contaminated soils. Generally, the remediation periods by bioaugmentation are usually short which may be attributed to the short life span of microbes especially in unfavorable conditions to them. Therefore as the CMD loses it’s inoculate potency due to prolong remediation periods, decrease in their bioremediation potential could be envisaged.

Conclusion

The need to seek for environmental friendly, cost effective and a short throughput for the remediation of petroleum products polluted soils cannot be overemphasized. This is for economic, environmental health and safety and adequate food production reasons. In this study, the use of locally produced chicken manure digestates for the enhancement of natural attenuation of petroleum products polluted soils was investigated. Specifically, the effects and efficacy of this organic stimulation on the removal of the DRO fractions from petroleum polluted soils were evaluated

Previous researchers have shown that when the level of petroleum soil pollution is higher than 5 %, effects of bioremediation would be insignificant particularly at short duration. But this study has shown that chicken manure digestate could enhance substantial removal of petroleum contaminants from pollution levels higher that the 5 % limit. Specifically, this study has revealed that: the use of chicken manure digestates could optimally enhance the removal of petroleum contaminants in petroleum polluted soils up to 10 %. The use of chicken manure digestates led to shorter remediation throughput during the removal of the DRO from petroleum polluted soils and was efficient at short remediation periods. When the remediation period is above 84 or 168 days, repeat application of the CMD is recommended.

Acknowledgement

The correspondence author, Oghoje S. U. is grateful to the Petroleum Technology Development Fund (PTDF) for funding his Ph.D. project, the source of this publication.

References

Abatenh, E., B. Gizaw, Z. Tesgaye and M. Wassie, 2017. Application of microorganisms in bioremediation - review Journal of Environmental Microbiology. 1(1), 2-9.

Abdulkadir, A., 2015. Measurement of unsaturated hydraulic conductivity of Alfisols in a long-term Experimental Plot with Minidisk Tension Infiltrometer in Samaru, Northern Guinea Savanna, Nigeria. Nigerian Journal of Soil and Environmental Research. 13, 9 -14.

Abdulsalam, S. and A. B. Omale, 2009. Comparison of biostimulation and bioaugmentation techniques for the remediation of used motor oil contaminated soils Brazilian Archives of Biology and Technology 52 (3), 747-754.

Adedokun, M. O. and A. E. Ataga, 2007. Effects of amendments and bioaugmentation of soil polluted with crude oil, automotive gasoline oil and spent engine oil on the growth of cowpea (Vigna unguiculata. L Walp). Science Research Essay. 2(5), 147-150.

Adekunle, I. M., 2011. Bioremediation of soils contaminated with Nigerian petroleum products using composted municipal wastes Bioremediation Journal. 15 (4), 230 – 241.

Adoki, A. and T. Orugbani, 2007. Removal of crude petroleum hydrocarbons by heterotrophic bacteria in soils amended with nitrogenous fertilizer plant effluents African J. of Biotech. 6, 1529 -1535.

Akoachere, J. T. K., Akenji, T. N., Yongabi, F. N., Nkwelang, G. and Ndip, R. N. (2008). Lubricating oil-degrading bacteria in soils from filling stations and auto- mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates Africa Journal of Biotechnology. 7(11): 1700- 1706.

Akpoveta, O. V., F. Egharevba, O. W. Medjor, O. K. Ize-Iyamu and E. D. Enyemike, 2011. Microbial degradation and its kinetics on crude oil polluted soil Research Journal of Chemical Sciences. 1(6), 8-14.

Amadi, A., A. Dickson and G. O. Maate, 1993. Remediation of Oil Polluted Soils. Effect of Organic and Inorganic Nutrients Supplements on the Performance of Maize Water, Air and Soil Pollution 55, 59 – 76.

Anoliefo, O. G. (2016). Man and his Evironment: Unsustainable Exploitation of the Land and the Dilemma of the Dog. 172nd Inaugural Lecture, University of Benin, Benin City: 1 – 94.

Atlas, R. M., 1981. Microbial Degradation of Petroleum Hydrocarbons. An Environmental Perspective. Microbiological Review. 45, 180 – 209.

Bing, C. and L. Kenneth,2019. Marine oil spills Preparedness and countermeasures. World Seas: an Environmental Evaluation. Second Edition.

Cerqueira, V. S., M. R. Peraalba, F. A. O. Camargo and F. M. Bento, 2014. Comparision of bioremediation strategies for soil impacted with petrochemical oily sludge International Biodeterioration and Bioremediation. 95, 338-345.

Chorom, M., H. S. Sharifi and H. Motamedi 2010. Bioremediation of a Crude Oil-Polluted Soil by application of Fertilizers. Iran. J. Environ. Health. Sci. Eng., 7(4), 319 – 326.

Colores, G. M., R. E. Macur, D. M. Ward and W. P. Inskeep, 2000. Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil Applied and Environmental Microbiology, 2959 – 2964.

Dadrasnia, A. and P. Agumuthu, 2013. Potential biowastes to remediate diesel contaminated soils Global Nest Journal. 15(4), 474-484.

Dias, R. L., L. Ruberto, A. Calabro, A. L. Balbo, M. T. Del Panno and W. P. Mac Cormack, 2015. Hydrcarbon removal and bacteria community structure in on-site biostimulated biopilensystems designed for bioremediation of diesel contaminated Antartic soil Polar Biology. 38, 677-687.

Dibble, J. T. and R. Bartha, 1979. Effects of environmental parameters on the biodegradation of oil spillages Applied Environmental Microbiology. 37, 72-78.

Egwunatum, O. K., 2015. Bioremediation of Crude Oil Contaminated Soil with Varegated Compost Formulations. Nig. J. Soil and Env. Res. 13, 141-146

Ekebafe, M. O. and P. O. Oviasogie, 2015. Assessment of soil physico-chemical properties and micronutrients as Influenced by the age of the Oil Palm. ChemTech Journal. 10, 87 – 92.

Falodun, E. J., E. I. Otamere and S. A. Ogedegbe, 2015 Agronomic performance of tomato (Lycopersicon Esculentum Mill) to different fertilizers rates. Nigerian Journal of Soil and Environmental Research. 13, 15 – 18.

Gomez, F. and M. Sartaj, 2014. Optimization of field scale biopiles for bioremediation of petroleum hydrocarbon contaminated soil at low temperature conditions by response surface methodology (RSM) International Biodeterioration and Bioremediation. 103-109.

Ijah, U. J. J., H. Safiyanu and O. P. Abioye, 2008. Comparative study of biodegradation of crude oil in soil amended with chicken droppings and NPK fertilizer Science World Journal. 3(2), 63-67.

Iren, O. B., N. M. John and E. A. Imuk, 2014. Effect of varying rates of pig manures and NPK (15:15:15) fertilizer on growth, nutrient uptake and yield of fluted pumpkin (Telferia Occidentalis Hook F) Nigerian Journal of Soil and Environmental Research. 12, 75 - 81.

Jorgensen, K. S., J. Puustinen and A. M. Suortti, 2000. Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles Environment Pollution. 107, 245-254.

Margesin, R., A. Zimmerbauer and F. Schinner, 2000. Monitoring of bioremediation by soil biological activities Chemosphere 40(4), 339-346

Millioli, V. S., E. L. C. Servulo, L. G. S. Sobral and D. D. Carvalho, 2009. Bioremediation of crude oil- bearing soil: Evaluating the effects of Rhamnolipid addition to soil toxicity and to crude oil biodegradation efficiency Global Nest Journal. 11, 2, 181-188

Mmom, P. C. and T. Deekor, 2010. Assessing the Effectiveness of Land Farming in the Remediation of Hydrocarbon Polluted Soils in the Niger Delta, Nigeria. Research Journal of Applied Sciences, Engineering and Technology. 2(7), 654-660

Molina-Barahona, L., R. Rodríguez-Vázquez, M. Hernández-Velasco, C. Vega-Jarquín, O. Zapata-Pérez, A. Mendoza-Cantú and A. Albores, 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues Applied Soil Ecology. 27(2) 165-175

Oelkers, E. H. and E. Valsami-Jones, 2008. Phosphate mineral reactivity and global sustainability Element. 4, 83-87

Oghoje, S. U. and J. E. Ukpebor, 2020. The effects and efficacy of chicken manure digestates on bioremediation of petroleum hydrocarbons polluted soils Nigerian Research Journal of Chemical Sciences. 8 (1), 331-337.

Oghoje, S. U., J. E. Ukpebor and E. E. Ukpebor, 2018. Green compost for enhancement of soil conditions and microbial accessibilty to hydrocarbon pollutants in soils ChemTech Journal. 13, 59-71.

Oghoje, S. U., J. E. Ukpebor, E. E. Ukpebor and C. Ejeomo, 2020. Comparison of the effects of two forms of organic stimulation on the bioremediation of monocyclic-aromatic hydrocarbon in soils Journal of Chemical Society of Nigeria. 45(3), 555-566.

Okieimen, C. O. and F. E. Okieimen 2005. Bioremediation of crude oil-polluted soil- Effect of poultry droppings and natural rubber processing sludge application on biodegradation of petroleum hydrocarbon. Environment Science. 12, 1 – 8.

Onwudide, S. U., D. O. Asawalam and A. O. Ano, 2016. Influence of Agro-wastes on soil properties, growth and yield of Maize (Zea Mays) in an acid ultisol of Owerri, Imo State Nigerian Journal of Soil Science. 26, 279 – 294.

Oshomoh, O. E. and B. Ikhajiagbe, 2015. An Assessment of physico-chemical qualities of soil around a Palm Oil Mill Effluent (POME) burrow pit in Irrua, Edo state, Nigeria ChemTech Journal. 10, 78 -86.

Osodeke, V. E. and N. E. Osondu, 2006. Phosphorus distribution along a toposequence of coastal plain sand parent material in Southeastern Nigeria Agricultural Journal. 1, (3) 167-171

Oyem, I. L. R. and I. L. Oyem, 2013. Effects of crude oil spillage on soil physic-chemical properties in Ugborodo community International Journal of Modern Engineering Research (IJMER). 3, (6) 3336 – 3342.

Radwan, S. S., D. Al-Mailem, I. M. El-Nemr and S. Salamah, 2000. Enhanced remediation of hydrocarbon contaminated desert soil fertilized with organic carbons International Biodeterioration and Biodegradation. 46, 129-132.

Raskin, I., R. D. Smith and D. E. Salt, 1997. Phytoremediation of materials: Using plants to remove pollutants from the environment Current Opinions in Biotechnology. 8, 221-226.

Semple, K. T., B. J. Reid and T. R. Fermor, 2001. Impact of composting strategies on the treatment of soils contaminated with organic pollutants Environmental Pollution. 112, 269-283.

Silvia- Castro, G. A., I. Uad, J. Gonzalez Lopez, C. G. Fandino, F. L. Toledo and C. Calvo, 2012. Application of selected microbial consortia combined with inorganic and oleophilic fertilizers to recuperate oil-polluted soil using land farming technology Clean Technology and Environmental Policy. 14, 719-726.

Sojinu, O. S., O. O. Sonibare, W. Ji-Zhong and E. Y. Zeng, 2009. Polycyclic aromatic hydrocarbons in Sediments and Soils from Oil exploration areas of the Niger Delta, Nigeria. Journal of Hazardous Materials. 174, 641-647.

Van Gestel, K., J. Mergaert, J. Swings, J. Coosemans and J. Ryckebore, 2001 Bioremediation of diesel oil-contaminated soil by composting with biowaste Environmental Pollution.,. 125 . 5 361-368.

Xiao-Dong, H. E., Y., J. Gurska, R. Bernard, B. Glick and M. Greenberg, 2005. A multi-process phytoremediation system for decontamination of persistent total hydrocarbons (TPHs) from soils. Microbial Journal. 81, 139-147.

Xin, B.-P., C.-H. Wu, C.-H. Wu and C.-W. Lin, 2013. Bioaugmented remediation of high concentration BTEX-contaminated groundwater by permeable reactive barrier with immobilized bead Journal of Hazardous materials. 244, 765-772.

In Memoriam - Prof. Dr. Ivan Atanasov Petkov

20 February 1931 – 06 November 2019

Professor Atanasov was a well renowned soil scientist with broad knowledge and grasp of broader agricultural and natural sciences.

Born in Bejanovo, Bulgaria in 1931, Professor Atanasov entered academia in 1950 as an undergraduate at the High Agricultural Institute in Sofia. He graduated in 1955 with distinction and spent the next four years as a practicing agronomist in the agricultural cooperative of his native village, Bejanovo.

In 1959 he returned back to the High Agricultural Institute, Sofia as an assistant professor in the faculty of Agronomy. In 1965 he was awarded the title of "Candidate of biological sciences" from the Lomonosov Moscow State University for his thesis (1962 – 1965) "*Composition and properties of the mechanical fractions of Typical Chernozem soils from the region of Kursk*" conducted under the supervision of Professor N.A. Kachinski in the department of Soil Physics and Amelioration.

Upon his return from Russia he continued to work in the High Agricultural Institute, Sofia and in 1972 he became a Docent (associated professor) in the department of Soil Sciences. In 1977 he was awarded "Doctor of agricultural sciences" for his work on "*Erosion and evolution of the mineral fraction of the Bulgarian soils*". He was the Dean of the faculty of Agronomy in the years 1976 - 1979.

In 1980 he took the seat of Professor in the department of Soil Sciences of the High Agricultural Institute in Plovdiv, Bulgaria.

In the years 1986-1990 he was the general director of the Centre for Applied Sciences and Information to the Ministry of Environment where he was responsible for environmental monitoring and the development of environmental quality standards. In 1990 he returned to the Agricultural University in Plovdiv and worked there until his retirement in 1999. In this period he held positions as head of department for Tropical Agriculture, and head of the department of Soil Sciences. He was also the Dean of the Swiss-Bulgarian faculty of organic agriculture.

Following his interests in environmental matters and sustainability, he established the non-for-profit Foundation Institute for Sustainable Development and remained its president and general manager until 2011.

Over his long and productive career he was the scientific supervisor of 19 PhD students and of more than 100 bachelor and master students. He authored over 200 scientific publications, monographs and textbooks. He led more than 30 national and international research projects in the area of soil sciences and acted as the editor of many conferences' proceeding from international soil conferences.

His principal areas of interest in the area of soil sciences were (1) change of the mechanical chemical and mineral content of soils due to erosion and soil formation; (2) change and balance of the chemical soil content due to intensive agriculture, (3) development of standards for soil contamination and soil protection.

Substantial part of his scientific work in the later professional years and after his official retirement was dedicated to the creation of legislation for soil protection and to establishing national standards for measurement of soil quality and soil pollution.

He was a member of the editorial board of the Bulgarian Journal of Agricultural Science (1994 – 2006). Between 1986 – 1988 he was a member of the managing board of the INFOTERRA network, a complement of UN Environmental Programme's Earthwatch Programme which plays an important part in fulfilling UNEP's global mandate of monitoring, assessment and dissemination of information on the environment, catalyzing and spurring others to action at international, regional and national levels.

Throughout his career Professor Atanasov's contributions to the agricultural sciences have been recognized with numerous awards, medals and honours: Golden medal from the Ministry of Environment of Bulgaria (1988), 1300 Years Bulgaria medal (1981) and the Cyril and Methodius order. He was member of the Bulgarian Academy of Sciences and Arts, member of the International Union of Soil Scientists and member of the Bulgarian Soil Society.

His work provided him also with many opportunities to travel across the world as part of different projects or for conferences and international meetings.

He was a firm believer in sharing his knowledge and inspiring the next generation of scientists and above all providing encouragement and guidance to many young scientists. Many of them will remember him as a true role model and mentor, but also someone who was always ready to share his experience and debate opinions, in a considerate and supportive tone, with the occasional well placed joke to liven up the spirits.

His long career in science was an excellent one, with many applied contributions and pioneering work in the area of agricultural sciences. He pioneered work on sustainable development in Bulgaria long before this became the urgent challenge of our current generations.

Professor Atanasov made a difference and leaves a legacy that will continue to shape the views and professional careers of those he thought and who worked with him over the years.

Prof. Dr. Rayna Dilkova at 90th years old

Prof. Dr. Rayna Dilkova is a Bulgarian soil scientist and a long-term leader of the Bulgarian Soil Science Society. Rayna Dilkova was born on December 20th, 1930, in Sofia. She graduated from the faculty of Agronomy at the Higher Institute of Agriculture in Sofia. Prof. Dr. Dilkova has spent her career at the Department of Soil Physics at the Institute of Soil Science “Nikola Poushkarov”, where she worked from 1949 until 1999.

Rayna Dilkova defended her PhD thesis in 1971 on “Soil aeration regime of leached smolnitza and leached cinnamonic forest soil and possibilities for its improvement by artificial structuring”. She was habilitated as a Professor of Soil Science in 1986 after a habilitation thesis on “Structure and aeration of the main Bulgarian soil units”.

Her long-term research activity includes outstanding work on important problems in agriculture, melioration, and ecology, in which she examined the natural, intrinsically genetic physical, hydraulic, and mechanical properties of soils in Bulgaria and their change as a result of anthropogenic activity. Prof. Dilkova is a lead author for some of the new methods for soil compaction prevention and improvement of soil quality. The scientific advantages of implementation of those methods in practice were acknowledged by a copyright certificate (1987).

Prof. Dr. Dilkova is author of new quantitative criteria for scientific investigations that objectively measure and assess: a) water stability of aggregates and soil structure; b) decreased productivity of the main agricultural crops due to difficult aeration in compacted soil root zones and seasonal soil waterlogging. For the latter, Prof. Dr. Dilkova defined critical thresholds for water and air-filled pores restricting the oxygen root supply. By applying common methodology for determination and evaluation of physical soil properties under non-cultivated and cultivated conditions, Prof. Dr. Dilkova assessed the susceptibility and degree of the anthropogenic physical deterioration of Bulgarian soils. The latter was her major national contribution to the “Global assessment of soil degradation (GLASOD)” project (ISRIC-UNEP-ISSS-FAO) for the preparation of the World Map of Human-induced Soil Degradation (1990).

Prof. Dr. Dilkova has published 113 scientific papers and research reports. Her monograph “Structure, physical properties, and aeration of Bulgarian soils”, published in

2014, presents the most complete database and assessment of the soil physical properties of the main virgin and cultivated soils in Bulgaria.

Prof. Dr. Dilkova was a long-term member and acting secretary (1997-2004) of the Scientific Council for Soil Science, Agrochemistry, and General Agriculture, at the Higher Attestation Commission in Bulgaria. She has participated in many national and international soil science forums. Prof. Dr. Dilkova was a lecturer at the Center for Qualification of Agronomists and at the Higher Institute of Agriculture and an advisor of national and international PhD candidates. Prof. Dr. Dilkova has also been a referee for many PhD and habilitation theses.

During the period of 1980-2013, Prof. Dr. Rayna Dilkova was the secretary, vice-president and president of the Bulgarian Soil Science Society. She significantly contributed to the successful organization of many national soil science conferences in which international scientists were also invited to participate. She is member of IUSS and the European Confederation of Soil Science Societies.

Happy Anniversary Prof. Dilkova

Prof. Metodi Teoharov
President of Bulgarian Soil Science Society

BULGARIAN JOURNAL OF SOIL SCIENCE®

INSTRUCTIONS FOR AUTHORS

Manuscript Submission

Submission of the manuscripts implies that they have not been published before nor are currently submitted or considered for publication in any other journals, which should be declared in **cover letter** by the authors.

The manuscripts must be submitted in electronic version to the Editorial e-mail address: ***Journal@bsss.bg*** **and** ***bulsoil@gmail.com***

Language

Manuscripts should be written in clear and grammatically correct English. British or American English spelling and terminology must be used. If English is not your native language we strongly recommend that the text of your paper be checked by a native speaker or a professional science editor before submission.

Types of Manuscripts

Bulgarian Journal of Soil Science publishes four categories of papers and the authors must indicate the category of their submission in the cover letter:

- Regular research papers must be based on results of original research. They will normally have a maximum length of 20 pages including tables, illustrations and references (on separate sheets).
- Research notes and short communications should be concise, focused on new results and data. They will be no longer than 5 pages, including the tables and figures.
- Review papers (invited or consulted with the Editor prior to preparation of the manuscript) with maximum length of 25-30 pages (according to the importance of the material).
- Book reviews and critical comments on articles and events up to 3 printed pages.

General presentation of the manuscripts

The manuscripts should be double-spaced (1800 characters per page), without section breaks, on A4 (210 x 297 mm) paper size, with margins of at least 2.5 cm at the top, bottom and sides. Lines should be numbered in the margins with a continuous numbering from the start of the manuscript. All pages should be numbered consecutively in the bottom, right-hand corner.

The manuscript should be arranged as follows:

Title Page

The title page should include:

- Full and short (running head) titles of the manuscript. The full manuscript title must be limited up to 20 words, and the short title – up to 7 words in small capitals;
- The name(s) of the author(s) in small capitals;

- The affiliate institutions(s) and address(es) of the author(s);
- The e-mail address of the corresponding author.

Abstract

Please provide an abstract up to 1500 characters that includes a clear statement of the research objectives, a short overview of the methods used, the most important results and a conclusion. No references and abbreviations should be used in the Abstract.

Key words

Please provide 4 to 6 key words, which can be used for indexing purposes. Avoid overlaps with the title.

Order of sections

The standard order of sections should be: Introduction, Material and Methods, Results, Discussion (or Results and Discussion), Conclusion, Acknowledgements, and References.

The content of the manuscript should be structured in accordance with the following criteria

1. **Introduction**. It should present a general framework of the topic in justifying why the authors have performed the study. State-of- art on the research topic has to be presented briefly to derive the novelty of the study and to show its relation to the preceding works. A distinct paragraph at the end of this chapter should state the objectives of the work.

2. **Materials and Methods**. This chapter should present detailed information of the place of research, as well as of data collection and analyses. Sufficient detail should be provided to allow the work to be reproduced. Methods already published should not be described in text but rather indicated by a reference.

3. **Results and Discussion**. It is recommended that results of the research are presented in a clear and concise manner. Information in charts, tables, photos or graphics should not be repeated in the text. This chapter should not contain a comparative assessment with similar papers or information on data analyses procedures. Also to make a comparative analysis between the results of the current research and those already published. This should not reiterate the results of the work but rather explore their significance. Published literature which is relevant to results and aim of the current study should only be cited.

4. **Conclusion**. This chapter or subsection of the discussion chapter should be concise and derived from the results of the research and the discussion presented. General statements are not allowed.

Scientific style

Please always use internationally accepted signs and symbols for units, SI units. Genus and species names should be in italics.

Text Formatting

- Use a normal, plain font (e.g. 12-point Times New Roman) for text and line spacing at 1.15.
- Use italics for emphasis.
- Use the automatic page numbering function to number the pages.
- Do not use field functions.
- Use the equation editor for equations.
- Save your file in *.docx format.

- Headings. Please use no more than three levels of displayed headings.
- Abbreviations should be defined at first mention and used consistently thereafter.
- Footnotes can be used to give additional information. Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.
- Acknowledgments (10-point plain font) of people, grants, funds, etc. should be placed in a separate section before the reference list. The names of funding organizations should be written in full.

Tables

The tables should always be cited in text in consecutive numerical order and must be numbered using Arabic numerals. Each table must be as simple as possible, double-spaced, of size no bigger than 12 x 18 cm. For each table, please supply a table caption (title) describing its content. Footnotes to tables should be indicated by superscript lower-case letters or asterisks and included beneath the table body.

Illustrations

All illustrations, including charts and photos, must be labelled as Figures. They must be numbered consecutively and should be provided with an explanatory legend on a separate sheet entitled ‘Figure captions’. They should conform to the size of the typed area (12 x 18 cm) which is the maximum size for the illustrations. Magnification should be shown by scale bars. Files in XLS, TIF, JPG and WMF formats are accepted. After the paper is accepted the editors will ask for figures in JPG or TIF format with 300 Dpi. Colour illustrations are accepted without fees for electronic publications. The editors reserve the right to decide what figures are justified and can be published in RGB colour (other figures will be removed or published in black and white).

References

Citation

Cite references in the text by name and year in parentheses as follows: Bauer (1932) or Bauer, Smith (1951) or (Bauer, 1930; Smith and Albert, 1931, 1935; Bauer et al., 1965) or Steffanic et al. (1951), up to two authors, give the names; for more than two authors, give the name of the first author followed by ‘et al.’. Check each reference with the original article and refer to it in the text. All authors of a paper should be cited in the list of references. References ‘in press’ shall only be cited when they have been accepted for publication.

Reference list

References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters ‘a’, ‘b’, ‘c’, etc., placed after the year of publication. In case of papers written in other than Latin letters, if there is an English (or German, French) title in the summary, it may be used. If there is not such a summary, the author’s name must be transcribed (using British English transcription) and the title of the paper must be translated into English. This should be noted in round brackets at the end of the paragraph, for instance: (In Bulgarian).

Some examples for references to journal publications, books, chapters in edited books and electronic publications or databases:

Journal articles

- Atanassova, I., S. Doerr, 2010. Organic compounds of different extractability in total solvent extracts from soils of contrasting water repellency. European Journal of Soil Science, 61, 298-313.
- Calderón, H. W., 1982. The content and composition of humus in arid alluvial soil of Peru. Soil Science, Academy of Sciences of USSR, Moscow, 8, 53-59. (in Russian).

Books

- Sparks, D. L., 2002. Environmental Soil Chemistry, 2nd Edition, Academic Press, San Diego, CA.

Book chapters or conference proceedings

Petrova, R., 1989. Loss of Humus Soil in the Process of Reclamation of Damaged Terrains. In: N. Sherbanova, R. Nacheva (Eds.). Proceedings from Fourth National Conference on Soil Science ‘Problems of Soil Science in Intensive Agriculture’. BSSS, N. Poushkarov Soil Science and Yield Programming Institute, Sofia, 203-206, (In Bulgarian).

Online publications

Atanassova, I., S. H. Doerr, 2011. Changes in Soil Organic Compound Composition Associated with Heat-Induced Increases in Soil Water Repellency. European J. of Soil Science, DOI: http://10.1111/j.1365-2389.2011.01350.x

Dissertations and Phylosophy Doctor Theses

Hristova, M., 2013. content and availability of microelements-metals in Technogenic soils. Ph.D. Thesis. Agricultural Academy, ISSAPP ‘N. Poushkarov’, Sofia, 140. (In Bulgarian)

General Peer-Review Process

The submitted manuscript should not have been previously published and must not be currently considered for publication in another journal. Violations of these rules will strictly result in an immediate rejection of the submitted manuscript.

Submission of Manuscripts

All manuscripts submitted for publication will be peer-reviewed by anonymous referees.

Submitted manuscripts will undergo a Plagiarism Check in the Editorial Office.

Papers will be registered with a date of submission and a date of acceptance for publication. If papers sent for revision are not returned to the editorial office within five weeks, these shall be registered with a new date of submission.

The corresponding author shall be notified in case a paper is rejected not later than two weeks after the decision had been taken.

The authors are responsible for the opinions expressed by them and for the plausibility of their data.

The authors must fill the "Copyright Release Form".

The Journal is free, web based and open access.

Only printed hard copy is not free, the price is 50 Euro, if it is required.